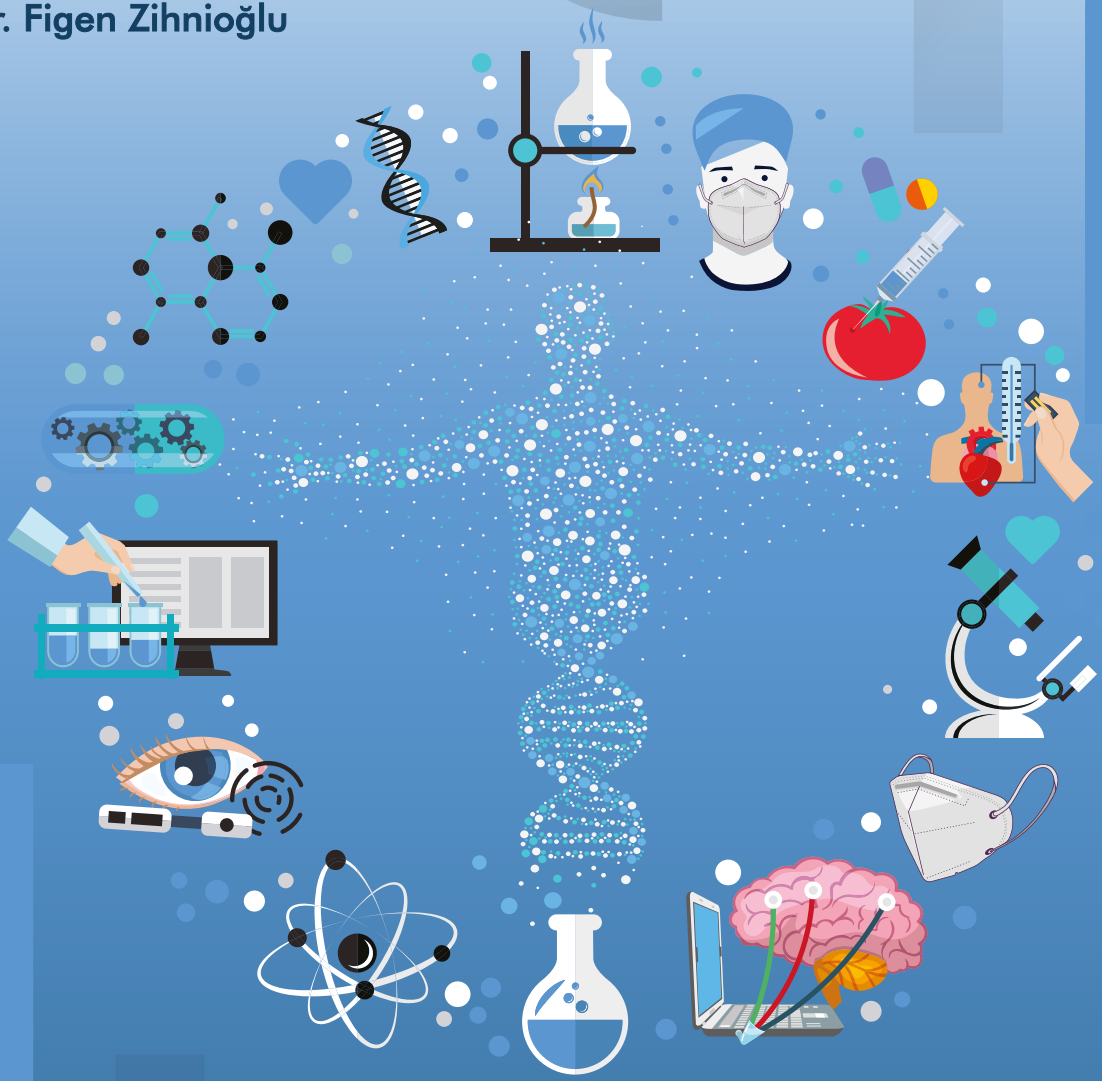
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Inflammation and *in vitro* Diagnostics

İnflamasyon ve *in vitro* Diyagnostik

Türkiye

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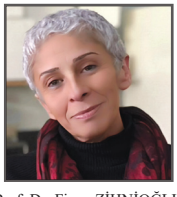
PREFACE ÖN SÖZ



Prof. Dr. Hilal KOÇDOR



Prof. Dr. Aysun PABUÇÇUOĞLU



Prof. Dr. Figen ZİHNİOĞLU

Perspectives on Inflammation in Preclinical and Clinical Studies and Current Monitoring: Integration of Inflammation-in vitro Diagnostic Diagnosis, Application, Devices, and Methods

Cancer, infection, and wound healing are three fundamental aspects of medicine, involving nearly all medical branches equally. Interestingly, these three conditions activate similar and common molecular pathways and cellular processes. In a sense, all these processes fall under the umbrella of "INFLAMMATION". Due to its complexity and broad scope, this topic has not been sufficiently compiled through common points. In this book, we aim to view diseases through the lens of inflammation.

Defined as a complex biological response of the organism to harmful stimuli, the "inflammatory response" can also occur in various conditions such as infections, tissue damage, or autoimmune diseases. Understanding and managing the inflammatory response is critically important in both preclinical and clinical studies. In recent years, in vitro diagnostic devices and methods have provided significant advances in the diagnosis and monitoring of inflammation. This book discusses the importance and application areas of integrating preclinical and clinical studies on inflammation with in vitro diagnostic devices and methods.

Preclinical research uses various animal models and cell culture systems to understand the molecular and cellular mechanisms of inflammation. These models are important for evaluating the efficacy and safety of drug candidates. Preclinical studies enable the discovery of new biomarkers that can be used for the early diagnosis and prognosis of inflammation. These biomarkers are thoroughly characterized before being validated in clinical studies.

Accurate and early diagnosis of inflammation in clinical studies plays a critical role in monitoring the course of the disease and the response to treatment. During this process, in vitro diagnostic methods and devices support clinical decisions by providing rapid and precise results at the patient's bedside. Patient-centered approaches in clinical studies allow for the development of personalized treatment strategies. These approaches are enriched with the data provided by in vitro diagnostic devices.

Significant technological advancements have been made in vitro diagnostic devices, including biosensors, microfluidic devices, and advanced imaging techniques in recent years. These devices enable the precise and rapid detection of inflammation. in vitro diagnostic devices offer a wide range of applications in laboratory settings. The detection of inflammatory biomarkers in blood, urine, or other biological samples is one of the most common uses of these devices.

The integration of preclinical and clinical studies with in vitro diagnostic devices and methods requires multidisciplinary approaches. This integration involves the collaboration of bio-

medical engineering, clinical medicine, bioinformatics, and other fields. The importance of inflammation in personalized medicine is increasing. Customized treatment approaches according to individual patient profiles become possible with the use of in vitro diagnostic data.

The current monitoring of preclinical and clinical studies on inflammation and the combined use of in vitro diagnostic devices and methods offer significant advantages in disease diagnosis and treatment. This integration contributes to the development of more effective and personalized approaches to patient treatment processes. In the future, technological advancements and multidisciplinary collaborations will further develop this field.

In this book, the integration of preclinical and clinical studies on inflammation with in vitro diagnostic methods is discussed in a multidisciplinary and comprehensive manner, examining inflammation from different perspectives, from basic research to clinical applications. By emphasizing the potential of this field, which offers innovative approaches to the diagnosis and treatment of inflammation, from research laboratories to clinical patient management, we hope to inspire and guide researchers, clinicians, and students.

We aim to contribute to the development of more effective treatment methods by providing the necessary knowledge and tools to understand and manage the complex nature of inflammation. By discussing the opportunities and challenges provided by the integration of preclinical and clinical studies with in vitro diagnostic devices and methods, we hope to lay a foundation for future research in this area.

We extend our gratitude to all researchers, clinicians, and health professionals who inspired and supported us in preparing this book. By sharing our knowledge in this field, we hope to open new horizons in the understanding and management of inflammation.

Respectfully.

Prof. Dr. Hilal KOÇDOR

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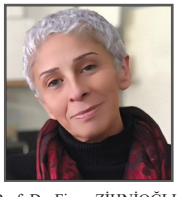
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Preklinik ve Klinikte İnflamasyona Bakış ve Güncel Takip;

İnflamasyon-in vitro Diyagnostik Tanı, Uygulama, Cihaz ve Yöntem Birlikteliği

Kanser, enfeksiyon ve yara iyileşmesi, tıbbın üç temel konusudur ve hemen hemen tüm branşları eşit derecede ilgilendirmektedir. İlginç bir şekilde bu üç durumda da benzer ve ortak moleküler yolaklar ve hücresel süreçler aktive olmaktadır. Bir anlamda bu süreçlerin tamamı "İNFLAMASYON" kapsamı altındadır. Bu konu, karmaşıklığı ve geniş kapsamı nedeniyle ortak noktalar üzerinden yeterince derlenmemiştir. Bu kitapta hastalıklara inflamasyon penceresinden bakmayı amaçladık.

Organizmanın zararlı uyaranlara karşı verdiği karmaşık bir biyolojik yanıt olarak tanımlanan "inflamasyon yanıtı"; enfeksiyon, doku hasarı veya otoimmün hastalıklar gibi çeşitli durumlarda da ortaya çıkabilir. İnflamatuar yanıtın anlaşılması ve yönetilmesi hem preklinik hem de klinik çalışmalarda kritik öneme sahiptir. Son yıllarda, in vitro diyagnostik cihazlar ve yöntemler, inflamasyonun tanı ve takibinde önemli ilerlemeler sağlamıştır. Bu kitapta, inflamasyonda preklinik ve klinik çalışmaların güncel takibi ile in vitro diyagnostik cihaz ve yöntemlerin entegrasyonunun önemi ve uygulama alanları ele alınmıştır.

Preklinik araştırmalar, inflamasyonun moleküler ve hücresel mekanizmalarını anlamak için çeşitli hayvan modelleri ve hücre kültürü sistemleri kullanır. Bu modeller, ilaç adaylarının etkinliğini ve güvenilirliğini değerlendirmek için önemlidir. Preklinik çalışmalar, inflamasyonun erken tanısı ve prognozunda kullanılabilecek yeni biyobelirteçlerin keşfine olanak tanır. Bu biyobelirteçler, klinik çalışmalarda doğrulanmadan önce kapsamlı bir şekilde karakterize edilir.

Klinik çalışmalarda inflamasyonun doğru ve erken teşhisi; hastalığın seyri ve tedavi yanıtının izlenmesi için kritik bir rol oynar. Bu süreçte, in vitro diyagnostik yöntemler ve cihazlar, hasta başında hızlı ve hassas sonuçlar sağlayarak klinik kararları destekler. Klinik çalışmalarda, hasta tabanlı yaklaşımlar, bireyselleştirilmiş tedavi stratejilerinin geliştirilmesine olanak tanır. Bu yaklaşımlar, in vitro diyagnostik cihazların sağladığı verilerle zenginleştirilir.

Son yıllarda, biyosensörler, mikroakışkan cihazlar ve ileri görüntüleme teknikleri gibi in vitro diyagnostik cihazlarda, önemli teknolojik ilerlemeler kaydedilmiştir. Bu cihazlar, inflamasyonun hassas ve hızlı bir şekilde tespit edilmesini sağlar. in vitro diyagnostik cihazlar, laboratuvar ortamında geniş bir uygulama yelpazesi sunar. Kan, idrar veya diğer biyolojik örneklerde inflamatuar biyobelirteçlerin tespiti, bu cihazların en yaygın kullanım alanlarından biridir.

Preklinik ve klinik çalışmaların in vitro diyagnostik cihaz ve yöntemlerle entegrasyonu, multidisipliner yaklaşımları gerektirir. Bu entegrasyon; biyomedikal mühendisliği, klinik tıp, bioinformatik ve diğer alanların iş birliğini içerir. İnflamasyonun kişiselleştirilmiş tıpta önemi

giderek artmaktadır. Bireysel hasta profillerine göre özelleştirilmiş tedavi yaklaşımları, in vitro diyagnostik verilerin kullanımıyla mümkün hâle gelir.

İnflamasyonda preklinik ve klinik çalışmaların güncel takibi ile in vitro diyagnostik cihaz ve yöntemlerin birlikte kullanılması, hastalık tanısı ve tedavisinde önemli avantajlar sunar. Bu entegrasyon, hastaların tedavi süreçlerinde daha etkin ve bireyselleştirilmiş yaklaşımların geliştirilmesine katkı sağlar. Gelecekte, teknolojik ilerlemeler ve multidisipliner iş birlikleri, bu alanın daha da gelişmesini sağlayacaktır.

Bu kitapta; inflamasyonda preklinik ve klinik çalışmaların takibi ile in vitro diyagnostik yöntemlerin entegrasyonu, multidisipliner ve kapsamlı bir şekilde ele alınmış, temelden kliniğe inflamasyon farklı bakış açıları ile irdelenmiştir. Araştırma laboratuvarlarından, klinik hasta yönetimine kadar geniş bir yelpazede, inflamasyonun tanı ve tedavisinde yenilikçi yaklaşımlar sunan bu alanın potansiyelini vurgulamıştır.

Kitabın; araştırmacılara, klinisyenlere ve öğrencilere ilham verici ve yol gösterici olmasını umut ediyoruz. İnflamasyonun karmaşık doğasını anlamak ve yönetmek için gerekli bilgi ve araçları sunarak, daha etkili tedavi yöntemlerinin geliştirilmesine katkı sağlamayı amaçlıyoruz. Kitap içeriğinde, preklinik ve klinik çalışmaların in vitro diyagnostik cihaz ve yöntemlerle entegrasyonunun sağladığı fırsatları ve karşılaşılan zorlukları tartışarak, bu alanda gelecekteki araştırmalar için bir temel oluşturmayı hedefliyoruz.

Okuyucularımıza, bu kitabı hazırlarken bize ilham veren ve destek olan tüm araştırmacılara, klinisyenlere ve sağlık profesyonellerine teşekkürlerimizi sunarız. Bu alandaki bilgi birikimimizi paylaşarak, inflamasyonun anlaşılması ve yönetilmesinde yeni ufuklar açmayı umuyoruz.

Saygılarımızla.

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Inflammation and *in vitro* Diagnostics

İnflamasyon ve *in vitro* Diyagnostik

CONTENTS - İÇİNDEKİLER

Physiopathology of Inflammation 1
İnflamasyonun Patofizyolojisi
Şevket RUACAN

Biochemistry of Inflammation: Its Mediators and Activities 6
İnflamasyon Biyokimyası: Mediyatörler ve Mekanizmaları
Alper Baran SÖZMEN, Eser Yıldırım SÖZMEN

***in vitro* Diagnostics Testing and Future Biochemistry in the Diagnosis of Inflammation** 13
İnflamasyon Tanısında *in vitro* Diyagnostik ve Geleceğin Biyokimyası
N. Nuray ULUSU

Classic and New Markers of Inflammation 18
İnflamasyonda Klasik ve Yeni Belirteçler
Ezgi EKİNCİ, Z. Günnur DİKMEN

Clinical Applications of Cytokines as Inflammation Markers: An Overview 28
İnflamasyon Belirteci Olarak Sitokinlerden Klinik Yararlanım: Genel Bakış
Sedef YENİCE

Sepsis Biomarkers 38
Sepsis Biyobelirteçleri
Ali Necati GÖKMEN, Ejder Kamil ÖZTÜRK

The Role of Nuclear Medicine in Clinical Molecular Imaging of Inflammation 44
İnflamasyonun Klinik Moleküler Görüntülemesinde Nükleer Tıbbın Yeri
Yeşim CEYLAN, Zehra ÖZCAN

Clinical Molecular Imaging in Inflammation with Radiological Perspective 50
Radyolojik Bakışla Yangıda Klinik Moleküler Görüntüleme
Oğuz DİCLE

New Approaches to Anti-Inflammatory Therapy: Nanotechnology Applications 56
Anti-İnflamatuar Tedavide Yeni Yaklaşımlar: Nanoteknoloji Uygulamaları
Sakine TUNCAY TANRIVERDİ, Özgen ÖZER

Inflammation and Immunity 66
İnflamasyon ve Bağışıklık
Haluk Barbaros ORAL

Inflammation and Microbiota 73
İnflamasyon ve Mikrobiyota
Özge Kamer KARALAR PEKUZ, Nur ARSLAN

Effects of Inflammation on Drug Metabolism and Pharmacogenetic Factors 80
İnflamasyonun İlaç Metabolizması Üzerine Etkileri ve Farmakogenetik Faktörler
İlter DEĞER, Elif BABAOĞLU, Melih Ö. BABAOĞLU

Metabolic Syndrome, Obesity and Inflammation 89
Metabolik Sendrom, Obezite ve İnflamasyon
Koray ATİLA

Identification of Endocrine Disruptors Via *in vitro* Diagnostic Tests: 94
Current Methods and Future Aspects
in vitro Diyagnostik Testlerle Endokrin Bozucuların Tanımlanması:
Mevcut Yöntemler ve Gelecek Beklentileri
N. Nuray ULUSU

Inflammatory Response in Wound Healing and Tissue Repair 99
Yara İyileşmesi ve Doku Onarımında İnflamatuar Yanıt
Özlem SİLİSTRELİ

Inflammation and Carcinogenesis 106
İnflamasyon ve Karsinogenez
Selman SÖKMEN, Berke MANOĞLU

Trauma and Inflammation 123
Travma ve İnflamasyon
A. Murat ÖZTÜRK, Arman VAHABİ

Gut and Inflammation 129
Bağırsak ve İnflamasyon
Göksel BENGİ, Nilay DANIŞ, Müjde SOYTÜRK

Inflammation in Rheumatoid Arthritis 139
Romatoid Artritte İnflamasyon
Haluk CİNAKLI, Servet AKAR

Aging and Inflammation 146
Yaşlılık ve İnflamasyon
Fatma Sena DOST, Açelya GÖKDENİZ YILDIRIM, Ahmet Turan IŞIK

Movement and Inflammation 151
Hareket ve İnflamasyon
Bilge KARA

Surgical Inflammation 157
Cerrahi İnflamasyon
İskender SAYEK

Idiopathic Granulomatous Mastitis 161
İdiyopatik Granülomatöz Mastit
Mehmet Ali KOÇDOR

Inflammation and Organ Transplantation 166
İnflamasyon ve Organ Transplantasyonu
Tarkan ÜNEK

Herbal Medicines with Anti-inflammatory Effect 170
Bitkisel Anti-İnflamatuarlar
Erdem YEŞİLADA

Biomarkers Used in the Diagnosis of Hyper-Inflammation of COVID-19 176
COVID-19 Hiper-İnflamasyonunun Tanısında Kullanılan Biyobelirteçler
Tanıl KOCAGÖZ

The Therapeutic Effect of Ozone and/or Prolotherapy on the Free Oxygen Radicals 180
Serbest Oksijen Radikalleri Üzerine Ozon ve Proloterapinin Tedavi Edici Etkisi
Selim KARABEKİR

Complementary Medicine Modalities: 190
The Effect of Acupuncture Treatment and Mesotherapy on Free Oxygen Radicals
Tamamlayıcı Tıp Modaliteleri: Akupunktur Tedavisi ve Mezoterapinin Serbest Oksijen Radikalleri Üzerine Etkisi
Nüket GÖÇMEN KARABEKİR

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PRESENTATION



Prof. Dr. Yahya Rauf LALELİ

This book, prepared by Türkiye Klinikleri, is a resource containing valuable information about what our immune system is and how it works, summarized by our esteemed scientists.

Inflammation is a word derived from the Latin word "inflammare", which began to be used in the seventeenth century, meaning to have the property of burning on its own. The word used for inflammation in Turkish is "yangı" and there is also the concept of burning in this word.

The immune system is a protection and defense system by which living things defend themselves against external factors, and the dimension discussed in this book is about inflammation, that is, the response of any foreign substance when it penetrates the living body and/or the reactive products of body, because this response is the key to survival.

In this book, current information, and methods regarding the body's responses to factors that cause sudden changes in the immune system, which can be any inorganic or organic factor, and the analysis of their results are discussed. The need to develop these methods is also emphasized. Although this resource, which provides topic headings for young scientists who will address these issues in the future, consists of articles dealing with in vitro methods, it is a guiding resource in which in vivo studies are also highlighted with visuals. I recommend that young scientists who evaluate this book through the electronic edition should consider the inter-chapter associations of parameters and concepts simultaneously.

We hope that this resource presented to our young scientists will ignite their curiosity and be a guide on their route.

TAKDİM

Türkiye Klinikleri tarafından hazırlanmış bu kitap; bağışıklık sistemimizin ne olduğunun, nasıl çalıştığının çok kıymetli bilim insanlarımız tarafından özetlendiği değerli bilgileri içeren bir kaynak.

Inflammation, on yedinci yüzyılda Latince'de kullanılmaya başlanan "inflammare" kelimesinden türemiş bir sözcük, kelime anlamı kendi kendine yanma özelliğine sahip olmak. Türkçe'de inflamasyon için kullanılan kelime ise "yangı" yine yanma kavramı var bu kelimede de.

Bağışıklık sistemi, canlıların kendilerini dış etkenlere karşı savunduğu bir korunma sistemi, bu kitapta ele alınan boyutu ise inflammation, yani herhangi bir yabancı maddenin canlı vücuduna girdiğinde ve/veya vücudun kendisinin verdiği cevap ile ilgili, çünkü bu cevap hayatta kalmanın anahtarı.

Bu kitapta bağışıklık sisteminde ani değişikliklere sebep olan etkenlere, ki bunlar inorganik, organik her türlü etken olabilir, vücudun verdiği cevaplar ve bunların sonuçlarının analizi ile ilgili güncel bilgiler, yöntemler ele alınıyor. Bu yöntemlerin geliştirilmesi gerekliliği de vurgulanıyor. Gelecekte bu konuları ele alacak genç bilim insanlarına konu başlıklarını veren bu kaynak, her ne kadar in vitro metotları ele alan makalelerden oluşsa da, in vivo çalışmaların da görsellerle vurgulandığı yol gösterici bir kaynak. Bu kitabı gençlerimizin elektronik baskı üzerinden değerlendirmelerini, parametrelerin ve kavramların bölümler arası ilişkilendirmelerini aynı anda ele almalarını öneririm.

Yeni yetişen bilim insanlarımıza sunulan bu kaynağın yol gösterici olması ümidiyle…

Physiopathology of Inflammation

İnflamasyonun Patofizyolojisi

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ABSTRACT Inflammation is the collection of cellular and molecular mechanisms which enables organisms to defend themselves against injurious agents, environmental changes and other organisms. All living creatures have mechanisms of inflammation. The primary target of inflammation is to fight injurious stimuli, restore physiologic systems and repair the damages. Throughout the ages inflammation have been studied by Hippocrates, Galen, Celsus and Virchow. In the 19th century Ehrlich and Metchnikoff have described cells responsible for inflammatory responses. Today the principal mechanisms of inflammation with cellular and vascular reactions have been elucidated. New techniques in imaging, ability to measure minute quantities of biological substances, and sophisticated molecular genetic methods have resulted in a much better understanding of inflammation and perhaps means to modulate it to cure diseases.

Keywords: Inflammation; immunity; leukocytes

ÖZET İltihap (yangı) organizmaların zararlı etkenler, çevre değişiklikleri ve diğer organizmalara karşı savunmak için geliştirdikleri mekanizmaların tümüne verilen addır. Tüm canlıların değişik iltihap yanıtları vardır. İltihabın temel hedefleri zedeleyici etkenlerle savaşma, fizyolojik sistemleri düzeltme ve zedelenmeleri onarma olarak özetlenebilir. Yüzyıllar boyunca Hipokrat, Galen, Celsus ve Virchow gibi öncüler iltihabı gözleme ve sınıflandırmaya çaba göstermişlerdir. 19. yüzyılda Ehrlich ve Metchnikoff iltihaptan sorumlu hücreleri belirlemiştir. Günümüzde iltihabın temel hücresel ve damarsal reaksiyonları açıklığa kavuşturulmuş; yeni görüntüleme teknikleri, çok düşük düzeydeki biyolojik molekülleri ölçme teknolojileri ve gelişmiş moleküler genetik yöntemlerle iltihabın çok daha iyi anlaşılması sağlanmıştır. İltihap yanıtının düzenlenmesi yoluyla hastalıkların iyileştirilmesi amacına yönelik çalışmalar da hızla ilerlemektedir.

Anahtar Kelimeler: İltihap; bağışıklık; lökositler

HISTORY OF INFLAMMATION STUDIES

All living creatures need to defend their organisms from external noxious stimuli for survival in hostile environments. From plants to unicellular organisms to mammalia increasingly complex systems of defense have developed through the evolution. In addition to acute mechanisms of inflammation, immune system with long term memory functions to defend the organism from continuous or repetitive insults.

Inflammation have been recognized and investigated by some of the earliest medical scientists. Smith Papyrus (3000-1600 BC) accurately describes cardinal signs of inflammation and attempts to discriminate it from neoplasia. Hippocrates, Galen, Celsus and more recently Virchow have each contributed to the identification of inflammatory changes in the body. These authorities described changes which are recognized as the **cardinal features of inflammation**:

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- Dolor (pain)
- Rubor (redness)
- Calor (increase in temperature)
- Tumor (swelling)
- Functio laesa (loss of function)

Following the invention of the microscope and definition of cell as the functioning unit of all living organisms cells specifically responsible from the inflammatory response were identified. Among many scientists studying different aspects of the inflammatory process, Paul Ehrlich and Elie Metchnikoff are particularly credited with description of the neutrophils and macrophages and for their contributions jointly received the Nobel prize in 1908.[1] Metchnikoff's observations on inflammation in various animal models are considered to be the starting point of many cell biology projects in our times.[2]

Other important contributions to the field include elucidating functions of the macrophages, opsonization, discovery of inflammatory mediators, interactions of inflammatory cells and the immune system, chemotaxis and cell migration and the role of inflammation in disease states.[2,3] More recently advanced imaging techniques, accurate measurement of intermediary molecules in tissue fluids, development of monoclonal antibodies against these mediators made possible to further understand the inflammatory process in health and diseases. Today the roles of various cell types, their functions, molecular aspects of cell migration, interactions among different populations of cells, the principal actions of mediators, adverse effects of inflammation have been mostly clarified.[4] Furthermore many drugs, antibodies and other treatment techniques have been developed to modulate the inflammatory response to reduce tissue injury. In the future we can expect the beneficial effects of inflammation to be increased while the harmful actions may be prevented in more rational treatment strategies.

CAUSES OF INFLAMMATION

Since inflammation is a general defense mechanism of the body many different agents may cause inflammatory responses. It must be emphasized that not all agents causing inflammation are external. Many internal events may result in inflammation, such as tissue necrosis secondary to ischemia or any other injurious effect. Additionally although inflammation is generally considered to be a beneficial defense mechanism in certain instances misdirected or excessive inflammation may be a cause of tissue damage. A typical example of inflammation-induced tissue damage is seen in autoimmune diseases where cells and tissues are injured by the actions of inflammatory cells or mediators. The main causes of inflammation is shown in Table 1.

ELEMENTS OF INFLAMMATION

Early investigators recognized that inflammation was mediated cells but a very important accompanying factor was the reactions of blood vessels. The roles of inflammatory cells was elucidated by Ehrlich, Metchnikoff, Conheim and von Recklinghausen in the 19th and early 20th Centuries. Furthermore these scientists observed that these cells were motile, moved towards the site of injury or inflammation, engulfed microbes etc. Additionally, it was observed that many vascular reactions occurred at the sites of inflammation such as dilatation, congestion, escape of fluid and cells out of the vascular spaces etc. Today we know that classes of inflammatory cells move towards sites of inflammation responding to chemical signals (chemokines), they pass through the walls of blood vessels by several molecular mechanisms, they secrete chemical mediators (cytokines) which influence other cells, causing them to multiply, move, cooperate and act to relieve inflammation and induce homeostasis. Some of the major agents of inflammation are summarized in Table 2.

Blood vessels: Some of the cardinal features of inflammation are directly secondary to changes in blood vessels, namely redness and increase in temperature due to increased blood supply and local congestion, swelling due to leakage of fluid from the vascular bed to the interstitial space (edema) etc. Local vascular changes at sites of inflammation include transient vasoconstriction of arterioles and dilatation of postcapillary venules. Especially retraction of endothelial cells leads to leakage of fluid and cells from the vascular space into the interstitial space and results in edema. These acute changes in the site of inflammation are brought about by chemical mediators, mainly

TABLE 1: Main causes of inflammation.

■ Infectious Agents
● Bacteria, viruses, fungi, parasites…
■ Physical Agents
● Heat, cold, trauma, pressure, radiation…
■ Chemical Agents
● Acids, alkali, toxins, drugs…
■ Immune Factors
● Foreign agents, autoimmunity, hypersensitivity..
■ Tissue necrosis

TABLE 2: Major agents of inflammation.

Blood vessels
Polymorphonuclear leukocytes
Macrophages
Lymphocytes: T, B, Natural killer
Plasma cells
Eosinophils
Mast cells
Mediators: cytokines, interleukins etc.

histamine and bradykinin, released from various cell types including endothelial cells, neutrophils and macrophages. In later stages of inflammation (repair and regeneration) blood vessels acquire new characteristics and start to give rise to new blood vessels (angiogenesis). These changes are induced by different mediators such as Vascular Endothelial Growth Factor (VEGF) which is secreted by a variety of cells and its main function is to induce proliferation and migration of endothelial cells.[2,4] As a result more blood containing nutrients and growth factors are transported to the injured site for repair and regeneration.

Cells: Earliest cells appearing at the site of inflammation are macrophages and neutrophils. Initially local tissue macrophages are attracted followed by infiltration by polymorphonuclear leucocytes extravasating from the blood vessels. The chemical signals which recruit these cells to the site come from endothelial cells, platelets, necrotic tissues and infiltrating cells themselves. In addition to these "early responders" cells of the immune system mainly lymphocytes are activated to mount an immune reaction to the invading microorganisms or foreign material. The mechanism by which inflammatory cells in the blood can respond to the damaged sites have been understood in recent years. This process occurs in several steps:

1. Margination: Following congestion and slowing of the blood flow the neutrophils assume a peripheral position in the blood vessel lumen and get closer to the endothelial cell layer.

2. Rolling and Leucocyte Activation: In the following sequence the neutrophil starts rolling on the endothelium, becomes activated and expresses surface molecules which bind to the molecules on the surface of the endothelial cells. In this process molecules such as selectins and cell adhesion molecules play important roles.

3. Arrest and Adhesion: Finally the movement of the neutrophil within the vascular lumen stops and a firm binding between the inflammatory cell and the endothelium takes place, again mediated by a variety of molecules including integrins and cell adhesion molecules.

4. Diapedesis: The process of migration of the inflammatory cell through the endothelial wall is called diapedesis. Recent studies demonstrated that neutrophils pass through spaces between endothelial cells (paracellular diapedesis) or through a pore in the endothelial cell cytoplasm (transcellular diapedesis).[5]

While the mechanisms of inflammation are developed to defend the organism against internal and external noxious agents, innate and adaptive immunity are also defensive mechanisms which operate for similar purposes. The cells responsible for the immune reactions are mainly lymphocytes and accessory cells. In chronic inflammatory processes immune system participates by providing humoral (antibodies) and cellular immune responses.

Macrophages and neutrophils are types of cells which are mainly responsible from neutralizing microorganisms, foreign material or necrotic cell debris. This specialized function is called phagocytosis.

During phagocytosis the inflammatory cell first identifies the offending agent (e.g a bacterium) through its surface receptors and binds it to the cell membrane. Subsequently the microbe surrounded by a vesicle of membrane is taken inside the cell (engulfment). This vesicle containing the microbe (phagosome) then binds to a lysosome in the cytoplasm forming a phagolysosome. In the next step the microbe is killed by the action of reactive oxygen species and nitric oxide within the vesicle and degraded by lysosomal enzymes.[4]

Phagocytosis is an important process in inflammation. Defects in phagocytosis result in inability to kill microorganisms and inefficient chemotaxis. Patients with abnormalities in phagocytic function may have recurrent bacterial infections as well as multiple other disorders. One typical example is Chediak-Higachi Syndrome associated with defective bacteriolysis due to inefficient phagolysosome formation.

Mediators: Inflammation in all steps depends on chemical substances known as mediators. Mediators may induce proliferation or inhibition of cells, may promote movement of cells towards the inflammatory sites (chemotaxis), vascular changes, coagulation etc. Some of the mediators in inflammation are shown in Table 3.

The sources and primary functions of mediators are shown in Table 4.

TABLE 3: Principal mediators of inflammation.

Cell-derived	Plasma protein-derived
■ Histamine	■ Complement products
■ Prostaglandins	■ Kinins
■ Leukotrienes	■ Proteases activated during coagulation
■ Platelet Activating Factor	
■ Reactive Oxygen Species	
■ Nitric Oxide	
■ Cytokines (TNF, interleukins…)	
■ Chemokines	

As can be seen in the table, mediators of inflammation are released from a variety of cell types and exert important roles in the functions of cells and progression of the inflammatory process. Many of these mediators are also active in many immunologic processes as well. Measurement of very small quantities of mediators in body fluids or even in cells has advanced our knowledge about the inflammation greatly. Furthermore development and use of specific drugs or monoclonal antibodies against these mediators made possible new interventions in clinical medicine to modify inflammatory responses and change of courses of many diseases.

Before we conclude this discussion on basic mechanisms of the inflammatory process two new concepts must be included.

Inflammasome: Up to this point we examined the roles of various inflammatory cells, their functions and modes of operation. In recent years, a unified concept of how inflammatory signals affect the cells and induce changes have been studied. Studies indicate that in response to inflammation a sensor/receptor system in the cells induces different proteins to form complexes in the cytoplasm of the cells and this initiates the cellular responses.[6] This protein complex is named inflammasome and acts in response to microbes and proteins of host origin. One main function of the inflammasome is to activate the enzyme system caspases which induce the formation of interleukin-1 derivatives and many local and systemic effects of inflammation. Studies indicate the role of inflammasomes in numerous disease processes such as degenerative diseases (Parkinson's, Alzheimer's, multiple sclerosis, …) and metabolic disorders (diabetes, atherosclerosis, obesity, …)

Neutrophil Extracellular Traps (NET's): Investigations on the functions of inflammatory cells revealed a special response of the neutrophils in response to certain inflammatory stimuli. Activated neutrophils may extrude nets composed of DNA-histone complexes and cytoplasmic material into the cellular media.[7] It is believed that these nets may entrap bacteria, fungi, protozoa and disseminate the inflammatory defense. Recently NET's are being studied for their roles in other inflammatory processes such as autoimmune diseases, coagulation disorders, diabetes and cancer progression and metastatic dissemination.

DISEASES AND INFLAMMATION

Inflammation is almost invariably present in infectious, neoplastic, degenerative and autoimmune diseases. However there are instances where inflammation may itself cause tissue destruction and promote disease progression. In some instances the body is unable to mount an adequate inflammatory or immune response and the defense mechanisms are impaired. Examples of diseases in which inflammation is a major component are given in Table 5.[8-17]

CONCLUSION

As can be seen from the discussion above inflammation is a very important physiological process protecting the organism from foreign microbes and damaging agents. An in-

TABLE 4: Principal mediators of inflammation, sources and primary functions.

Mediator	Source	Functions
Histamine	Mast cells, basophils, platelets	Vasodilation, increased permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased permeability, chemotaxis, leukocyte adhesion and activation
Cytokines	Macrophages, endothelial cells	Endothelial activation, fever, metabolic abnormalities, hypotension
Chemokines	Leukocytes, macrophages	Chemotaxis, leukocyte activation
PAF	Leukocytes, mast cells	Vasodilation, increased permeabilitiy, leukocyte adhesion, chemotaxis…
Complement	Plasma (produced in liver)	Chemotaxis, leukocyte activation, direct target killing, vasodilation
Kinins	Plasma (produced in liver)	Increased permeability, smooth muscle contraction, pain

TABLE 5: Examples of diseases with prominent inflammatory component.

Infections	Liver Diseases
Injuries	Hepatitis
Lung Diseases	Gastrointestinal Diseases
Pneumonias	Inflammatory bowel diseases
Heart Diseases	Musculoskeletal Diseases
Ischemic	Myositis
Inflammatory	Rheumatic diseases
Kidney Diseases	Central Nervous System Diseases
Glomerulonephritis	Encephalitis, meningitis
Skin Diseases	Multiple sclerosis
Dermatitis	Parkinson, Alzheimer
	Psychiatric diseases (depression)

flammatory process accompanies almost all diseases and in most instances helps the organism in avoiding cell and tissue damage and eliminating the offending agent. However in some instances the inflammation itself may be the cause of damage as in the case of autoimmune diseases. In recent years principal mechanisms of inflammation have been studied and important information on vascular changes, cellular actions, roles of mediators have been elucidated. By understanding the mechanisms pharmacologic and immunologic agents have been developed to check the inflammatory response to aid in healing and to prevent damage of cells and tissues. We hope the studies with advanced biochemical, molecular biologic and immunologic methods will yield further information to monitor and direct inflammation for more rational methods of treatment of diseases in future.

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Biochemistry of Inflammation: Its Mediators and Activities

İnflamasyon Biyokimyası: Mediyatörler ve Mekanizmaları

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ABSTRACT Inflammation is a process which aims to protect the host against bacteria, viruses, and infections; it is executed by both immune and non-immune cells. Acute inflammatory response is mainly driven by mast cells which release histamine, bradykinin, Tumor necrosis factor (TNF) and Interleukin-1 (IL-1) subsequently resulting in vasodilation by nitric oxide and vascular leakage of leucocytes and activation of complement and clotting system. The foreign agent undergoes phagocytosis and is rendered harmless by increased concentration oxygen radical and nitric oxide. However, various factors such as aging, obesity, stress, environmental factors lead to low-grade "sterile" induction of inflammation and it's defined as chronic systemic inflammation (CSI). Pathogen-associated molecular patterns (PAMPs) and/or Damage-associated molecular patterns (DAMPs) activate inflammatory response by binding to Pattern Recognition Receptors (mannose binding receptor, scavenger receptor, Toll-like receptor, G-protein receptor etc). The main distinction of CSI from acute inflammatory response is that CSI involve the immune attack and activated macrophages. Classically activated macrophages release many cytokines, adhesion molecules, growth factors, and matrix metalloproteinases. Altogether these effects might lead to tissue damage and result in chronic inflammatory diseases.

Keywords: Inflammation; pathogen-associated molecular patterns (PAMPs); damage-associated molecular patterns (DAMPs); nuclear factor kappa B

ÖZET İnflamasyon, konakçıyı bakterilere, virüslere, enfeksiyonlara karşı korumayı amaçlayan bir süreçtir, immün ve immün olmayan hücreler tarafından gerçekleştirilir. Akut inflamatuar yanıt, esas olarak histamin, bradikinin, TNF ve IL-1 salgılayan mast hücreleri tarafından yönlendirilir ve ardından nitrik oksit ile vazodilatasyon ve lökositlerin vasküler sızıntısı ve kompleman ve pıhtılaşma sisteminin aktivasyonu ile sonuçlanır. Yabancı madde fagositoza uğrar ve artan oksijen radikali ve nitrik oksit tarafından zararsız hale getirilir. Ancak yaşlanma, obezite, stres, çevresel faktörler gibi çeşitli faktörler inflamasyonun düşük dereceli "steril" indüksiyonuna yol açar ve bu kronik sistemik inflamasyon (CSI) olarak tanımlanır. İnflamasyon sürecinde, Patojenle ilişkili moleküler modeller (PAMP'ler) ve/veya Hasarla ilişkili moleküler modeller (DAMP'ler), Patern Tanıma Reseptörlerine (mannoz bağlayıcı reseptör, çöpçü reseptör, Toll benzeri reseptör, G-protein reseptörü vb.) bağlanarak inflamatuar yanıtı aktive eder. Kronik steril inflamatuar yanıtın, akut inflamatuar yanıttan ana farkı, bu inflamasyon sürecinin bağışıklık sisteminin uyarılması ve aktive edilmiş makrofajları içermesidir. Klasik olarak aktive olan makrofajlar birçok sitokin, adezyon molekülü, büyüme faktörü ve matriks metalloproteinazları serbest bırakır. Bu etkilerin tamamı dokularda hasara yol açarak kronik inflamasyon ile karakterize olan hastalıklar ile sonuçlanabilir.

Anahtar Kelimeler: İnflamasyon; patojen ilişkili moleküler yapılar; hasarla ilişkili moleküler yapılar; nükleer faktor kappa B

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In recent years, inflammatory events have been demonstrated to be involved in the pathogenesis of numerous acute and chronic diseases. The objective of this article is to discuss the mediators involved in the biochemical mechanism of inflammatory processes and their functions in the context of the literature. In the article, inflammatory events are classified as acute and chronic according to the duration of development, and

the functions of mediators are explained in this context. In the final section of the article, biomarkers that have been demonstrated to serve as indicators of inflammation in serum/plasma in the literature are elucidated

1. ACUTE PHASE OF INFLAMMATION

Inflammation is a process which aims to protect the host against any agents (i.e. bacteria, viruses, infections, burns, lacerations, trauma, radiation, chemicals etc) that damage in host cells. Inflammation processes are mainly executed by immune and non-immune cells (Table 1). The response of host so called acute phase of inflammation occurs in 4 successive stages: vasodilation, adhesion, chemotaxis and elimination of foreign molecules via phagocytosis. The goal of the organism is to localize and remove the agent, that's why at the beginning of inflammation vasoconstriction happens in the region, which lasts for a few seconds and afterwards, vasodilation occurs with the effect of histamine and nitric oxide released immediately after the vasoconstriction. Activation of mast cells and complement system occurs simultaneously in the region. With the effect of cytokines such as histamine, bradykinin, platelet activating factor (PAF), Tumor necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) that are released by mast cells in the region, acute phase proteins (Serum amyloid A (SAA), C reactive Protein (CRP), fibrinogene, α1-antitrypsin, haptoglobin, ceruloplasmin) increase and platelet activation occurs. Serotonin is released by activated thrombocytes, induces platelet aggregation and the coagulation cascade is stimulated at the site of vascular damage. The complement system, which is activated with the effect of anaflatoxin, opsonin, and MAC, stimulates three main events: 1) it causes contraction of entothelial cells, causing vasodilation and an increase in vascular permeability to ease the passage of incoming leucocytes to leave the vessel. There is also an increase in nitric oxide production with eNOS activation in endothelial cells. 2) it stimulates the cyclooxygenase and lipoxygenase pathways by activating the arachidonic acid metabolic pathway in the cell membrane. While 5-HETE and LTB4, which are produced in the lipoxygenase pathway, increase chemotaxis, LTE4 causes an increase in vascular permeability. Prostacyclins, whose production is increased in the cyclooxygenase pathway, and mediators such as PGF2α cause vasodilatation. 3) Finally, it leads to increased radical production.[1,2]

Different type of adhesion molecules participate in adhesion and chemotaxis process: the selectins, integrins, vascular addressins and lymphocyte homing receptors. Selectins which are expressed on cell surface of leukocytes (L-selectin), endothelial cells (P-selectin, E-selectin), and platelets (P-selectin), play a main role in binding of immune cells on endothelial cells. While L-selectin facilitates the interaction between leukocytes and endothelial cells, P-selectin facilitates binding of the PMNs, platelets on endothelial cells. E-selectin expression is upregulated by cytokines and bacterial cell wall products and facilitates the binding of leukocytes at sites of inflammation. The integrins which are expressed on surface of leucocytes, have a main role in migration of leukocytes through the vasculature to sites of injury and inflammation. Receptors on surface of integrins which are activated by selectins and cytokine/chemokines, can bind to its ligands namely intercellular adhesion molecule-1 and 2 (ICAM-1, ICAM-2), vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells.

Macrophage-1 antigen (MAC-1) binding is important in the adherence of monocytes to endothelial cells and also serves as a complement receptor to enhance the phagocytosis of opsonized cells or bacteria.[1-3]

Following adhesion and chemotaxis of neutrophils, the last stage of acute inflammation which covers phagocytosis, activation of proteases and proteinases, stimulation of radical production, becomes active. As it's clearly

TABLE 1: Main differences between acute and chronic inflammation.

	Acute	Chronic (Non-infective- Sterile)
Inducer/Factors	Bacterias, parasites, viruses, helminths, travma, radiation, chemicals	Stress, obesity, hypertension, insulin resistance, diet, sedanter life, aging, UV radiation, pesticides
Main Cells	Mastcells,thrombocytes, polymorph nuclear leucocytes (neutrophils, basophils, eosinophils). endothelial cells	Neutrophils Monocytes-macrophages Microglial cells
Duration	Hours	From days to years

known that phagocytosis is an associated with activation of lysosomes. This process is characterized by stimulation of oxidase enzymes [NADPH oxidase and myeloperoxidase (MPO)] and inducible nitric oxide synthetase (iNOS). The products of these type of enzymes in terms of superoxide anion radicals by oxidase enzymes and nitric oxide by iNOS, produces peroxynitrite which is toxic both for foreign cells and host cells. Besides production of superoxide radicals, MPO has a key role in inflammatory process through by inducing matrix metalloproteinases, stimulating p-selectin and inhibiting eNOS.[4,5]

Monocytes which migrate to the lesion area in 24-48 hours subsequently differentiate into macrophages. Two main different population of macrophages which were transformed from monocytes, have distinct characteristics and effects. Although it has not been clearly known the trigger factor for polarization type of macrophages, several molecules have been implicated in this process.[6] While microbial products, lipopolysaccharides (LPS), GM-CSF and IFN-γ induce transformation into classically activated macrophages (M1), helminths and other cytokines such as CSF-1, IL-4, IL-10, TGF-β, and IL-13 induce transformation into alternatively activated macrophages (M2). M1 macrophages are defined as pro-inflammatory macrophages due to their ability to secrete cytokines such as IL-1β, TNF, IL-12, IL-18 which are exacerbate inflammatory processes.[6,7] On the other hand, M1 macrophages increase nitric oxid production through inducing nitric oxide synthetase (iNOS) and shows antimicrobial effect by acidifying phagosomes.[6,7]

Inflammatory response to all agents that harm the body can end in one of the three paths: resolution, partial healing (healing with scar-fibrosis), spreading to the body. Following infection, production of antiinflammatory lipid mediators such as lipoxins, resolvins, maresins and protectins, production of antiinflammatory cytokines such as TGF-β and IL-10, inhibition of TNF-α production and apopitosis then contribute to the resolution of inflammation. Beside of TGF-β1 and TGF-α macrophages and epithelial cells release other growth factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) to facilitate healing of tissue.

2. CHRONIC SYSTEMIC (STERILE) INFLAMMATION

If the inflammatory process becomes persist due to lacking of resolution and/or presence of persistent inducer of inflammation by especially environmental and biological factors, upregulation of immune components leads to chronic systemic inflammation which is defined as low-grade, non-infective (that is, 'sterile') systemic chronic inflammation (SSCI) (Table 1).[8]

Recently, it has been shown that chronic systemic inflammation is responsible for the pathogenesis of many chronic diseases; such as type2 diabetes, obesity, atherosclerosis, metabolic syndrome (includes the triad of hypertension, hyperglycemia and dyslipidemia); hypertension; chronic kidney disease; cancer; mood disorders; neurodegenerative and autoimmune diseases; osteoporosis etc.[3,8-13]

The most important difference between acute inflammation and chronic systemic inflammation is the difference in the molecules and factors that induce inflammation. While acute inflammation is induced by infectious agents defined as pathogen-associated molecular patterns (PAMPs) and/or their antigenic determinants (LPS from gram negative bacterias, lipoteichoic acid and proteoglycans from gram + bacterias, mannose including carbohydrates, flagellin, non methyllated CpG, viral double-stranded RNA, bacterial DNA, etc.), SSCI is induced by agents originating from damaged cells called damage-associated molecular patterns (DAMPs).[8,14,15]

When factors that can cause SSCI in cells were investigated: senescence was shown to be an important contributor. Continuous immune stimulation might cause a steady pro-inflammatory state called inflammaging, which leads to a low grade, sterile and chronic inflammation. The reason or reasons of this state could be a variety of conditions, such as immunosenescene which is the remodelling of immune cells through aging. It has been shown that with aging, level of proinflammatory cytokines increase in blood which is considered to be in relation with a low-grade inflammation caused by inflammaging.[16,17] Besides increased cytokine levels, imflammaging is characterized reduced anti-inflammatory adipocyte tissue-derived adipokine/cytokine production. Therefore M1 macrophage polarization is more common in aging.[17]

The non-endogenous factors which promote SSCI include chronic infections, physical inactivity, microbioata dysbiosis, diet (relatively low in fruits, vegetables and other fiber- and prebiotic-rich foods and high in refined grains, alcohol and ultra-processed foods), social and cultural changes (physiological stressors in work life and/or famliy life, exposure to blue light, low social interactions) and environmental and industrial toxicants.[8,18] Another important factor is aging which was named as inflammaging ,is characterized by promoting of M1 polarization of macrophages and by increasing expressions of proinflammatory cy-

tokines (IL-1β, IL-18, and TNF-α.[17,19] Especially under the stress of ageing and obesity specific adipokines and chemokines are synthesized by adipose tissue further augmenting inflammation profile.[17]

Pattern recognition receptors (PRRs) family, which are expressed in immune cells such as macrophages, dendritic cells, might recognize both PAMPs and DAMPs, subsequently initiate innate immune response. PRRs were classified according to the organel where they showed their activity. While TLRs (Toll-like receptor) and C type lectin receptors were localized in membranes, RIG-I like receptors (Retinoic acid inducible gene-I) and NOD-like receptors (Nucleotide oligomerization domain) were localized in cytosol.[14,15]

RIG-I like receptors recognize viral nucleic acids and induce gene expression of IFN-β and NOD-like receptors that recognize the components from viral, bacterial and parasites induce NF-κB.

Toll like recepors (TLRs) which consisted of ten members (TLR 1, 2, 4, 5, 6, and 10 are localized in cell surface, TLR 3, 7, 8,and 9 are localized to endoplasmic reticulum, endosome and lysosome), recognize and bind PAMPs and endogenous ligands, then trigger a signal mechanism.[20]

Carbohydrate-like Leptin Receptors (CLRs) which were expressed in macrophages and dendritic cells, recognize carbohydrate moiety on surface of microorganisms. The ligands of mannose receptors that are a group of CLR, are lysosomal enzymes and myeloperoxidase. Dendritic cell-associated C type lectin (Dectin-1 and Dectin-2) receptors which are another group of CLR, have different ligands and function in immun response. Dectin-1 can bind the beta-1,3-glucans on the fungi and activates a signal transduction pathway. Dectin-2 recognizes alpha mannan on cell wall of fungi and lipoglycans from bacterias.

As a result of binding to PRR receptors, 4 main signaling pathways become activated (Figure 1).[2,4,9,20-23]

1. NFκ-B signalling
2. Mitogen activated protein kinase (MAPK) signalling
3. TRAF family member asscociated NFκB activator binding kinase (TBK1-RIF3) signalling
4. The inflammasome signalling

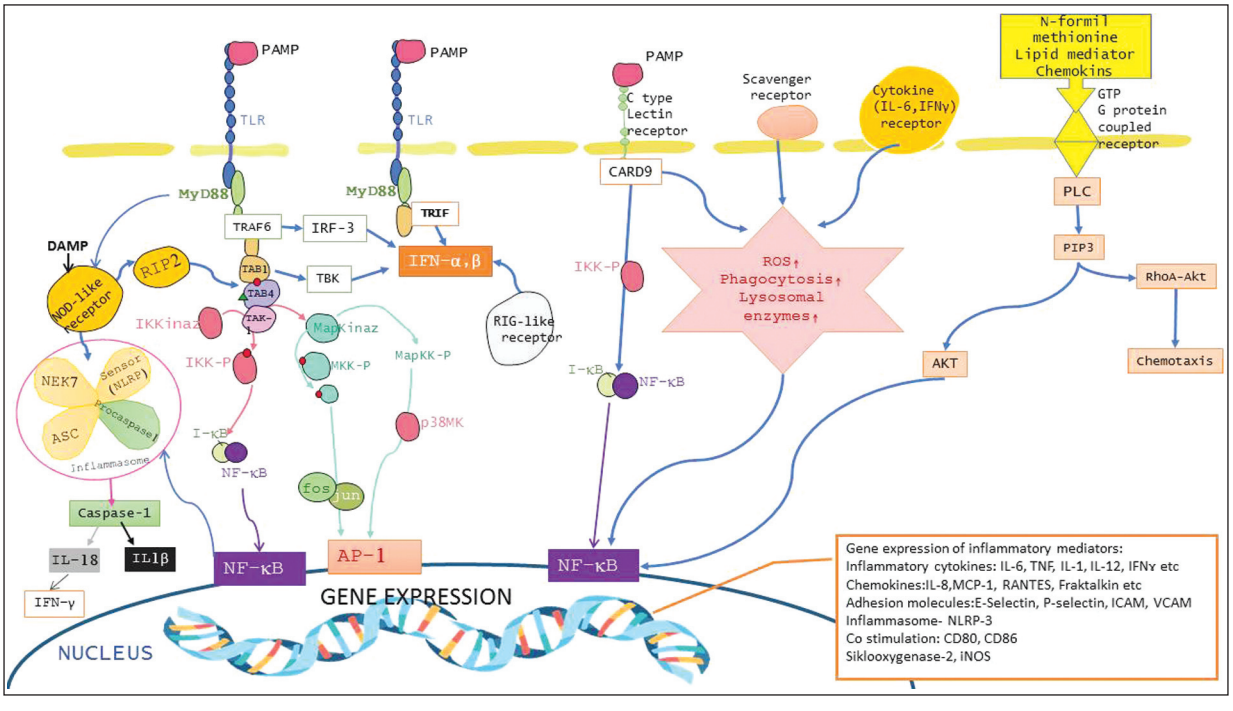


FIGURE 1: Biochemical mediators and signallling in chronic systemic inflammation.

AP-1: Activator Protein-1 ASC: Apoptosis-associated speck-like protein CARD9: Caspase recruitment domain family member 9; IRAK: IL-2 receptor associated kinase; IRF-3: Interferon regulatory factor-3; MAPK: Mitogen activated protein kinase; MyD88: Myeloid differentiation primary response gene 88 NEK7: NIMA-related kinase 7 NLRP3: NLR family pyrin domain containing 3 NOD: Nucleotide-binding oligomerization domain; PIP3: Phosphatidyl Insitol-3-phosphate; PLC: Phospholipase-C; RIG: Retinoic acid inducible gene; TAB-1 and TAB-4: TAK binding proteins; TAK-1: TGF beta activated kinase; TBK-1: TRAF family member associated NFKB activator binding kinaz; TLR: Toll-like receptor; TRAF: TNF receptor associated factor-6.

NFκB signalling pathway: NFκB is a transcription factor for inflammatory genes and plays a main role in inflammation and immune response. In order to stimulate gene transcription, it must be released from inhibitory protein (IκB) and must be translocated into nucleus. In Myd88 (Myeloid differentiation primary response gene 88) dependent pathway, TLRs transmit signalling to Myd88 through by binding Myd88's C terminus and then Myd88 activates IRAK 4 (IL-1 related kinase 4), subsequently IRAK-1 and IRAK-2. These kinases stimulates TRAF-6 (TNF-receptor associated factor-6) which induce formation of a complex including transforming growth factor-β-activated kinase-1 (TAK-1), TAK binding proteins (TAB-1 and TAB-4). This complex releases NFκB, by phosphorylation IκB kinase complex. It also activates activator protein-1 (AP-1) and interferon regulatory factor-3 (IRF-3).

In Myd88 independent pathway, TIR-domain containing adaptor protein inducing IFN-β (TRIF) is activated by TLR-3 and TLR4, and stimulates interferon type-1.

NOD-like receptors are activated by bacterial components inside the cell, and it activates RIP-2 and then TAK-1/TAB-1 complex which in turns activates transcription of NFκB.

Although RIF-I and MDA5 receptors are activated by binding to viral RNA, follow the similar signalling pathway which were ended by activation of NFκB through by induction of TRAF-3 and TRAF-6.

MAPK (mitogen activated protein kinase) signaling pathway: Unlike the NFκB signaling pathway, is activated by many extracellular stimuli (neurotransmitter, hormones, cytokines etc). Intermediate molecules in the Myd88-dependent pathway are the same as in the NFκB pathway, resulting in activation of NFκB. TRAF-6, which is activated in this pathway, also activates MAP kinases (JNK-c-jun N terminal kinase, p38 MAPK), causing the release of proinflammatory cytokines.

The TBK1-RIF3 (TRAF family member asscociated NFκB activator binding kinase) signalling pathway: TRIF (TLR3/TLR4-TIR domain containing adaptor protein-inducing interferon β) ve RIG-I/MAVS activate IRF-3 which is a transcription factor for synthesis of IFN-type1 and antiviral innate immune response.

The inflammasome signalling pathway: Inflammasome which is a protein complex, contain 4 parts; NEK, ASC, caspase protease, and a sensor receptor. The complex is activated by the binding of PAMPs or DAMPs to sensor protein in inflammasome (nucleotide-binding oligomerization domain (NOD)- and leucine-rich repeat (LRR)-containing receptors (NLRs), the absent in melanoma-2 (AIM2)-like receptors (ALRs) and pyrin protein). Procaspase-1 is activated when it is seperated from the structure. Caspase-1 plays an important role in the innate immune system by activating IL-1 beta and IL-18. IL-1 beta causes NFκB activation, also it stimulates apoptosis via MAPK pathway. IL-18 stimulates production of IFNγ and plays a main role in adaptive immunity.

The common end products of most of the signaling pathways listed above are Activator Protein-1 and activated NFκB, which causes the release of many proinflammatory cytokines as transcription factors.

Nuclear factor-κB (NF-κB) which is found cytoplasm as inactive in the resting state, is a transcription factor. It has a dimer structure which is consisted of 5 five related proteins. Upon phosphorylation of these proteins by IκB kinase (IκK) complex, the IκB undergoes proteasomal degradation thus free NFκB move into the nucleus.[21] NFκB regulates more than a hundred gene expression especially proinflammatory cytokines by binding to specific sites of DNA. (NFκB Target Genes » NF-κB Transcription Factors | Boston University (bu.edu) Another important transcription factor is Activator Protein-1 (AP-1) which involves in cell differentiation, survival, apoptosis, proliferation, cell transformation, and migration. AP-1 family of transcription factors includes Jun, Fos, ATF, and MAF as heterodimer or homodimer.[22]

NFκB and AP-1 are stimulated by distinct mechanisms, on the other hand NF-κB and AP-1 may affect each other's actions (Figure 1).

3. SERUM/PLASMA MARKERS OF INFLAMMATION

While clinical findings (heat, pain, redness, or swelling symptoms) are very evident in acute inflammation, it is very difficult to define chronic sterile systemic inflammation with clinical findings. Recent studies have shown that chronic inflammation is the basis of various chronic diseases (such as metabolic syndrome, Diabetes Mellitus, neurodegenerative diseases) with very diverse clinical features. Considering the molecular pathways mentioned above regarding the chronic inflammation process, also the adaptive immune response and cellular pathways and released molecules that are not mentioned in this section, it is not possible to talk about a single molecule that can indicate the chronic systemic inflammatory response.

In the studies performed, the number and distribution of leukocytes, leukocyte types and acute phase proteins

have been revealed as markers of both acute and chronic inflammation, but in this section, only the results of studies on chronic sterile systemic inflammation as well as innate immunity are summarized.

Kigka et al who reviewed the literature for diagnostic inflammatory markers of carotid artery disease (CAD), observed hs-CRP, the PTX-3, IL-6, and TNF-α as useful biomarkers for the diagnosis of CAD as well as monitoring of the disease progression.[24] Kolodziej et al. evaluated the prognostic role of MPO activity as an inflammatory marker in patients having acute coronary syndrome. The data from this meta-analysis which covers 4048 sources, showed that MPO determination might add value to risk assesment of patients having acute coronary syndrome. They proposed that predictive value of MPO is more specific in patients with STEMI (ST segment elevation myocardial infraction) and high-risk NSTEMI.[25] A study on children with familial hypercholesterolemia which investigated predictive value of inflammatory markers in early atherosclerosis, suggested, YKL-40 and myeloperoxidase were the more predictable markers of atherosclerosis even in early ages (<6 years old) than other classical inflammation markers such as hs-CRP, IL-6 and TNF-α.[26]

In a systematic review, results of 623 studies with inflammatory markers in metabolic syndromes were compiled, due to this rewiev, Rocha et al. stated that IL-6, IL-10, IL-18, TNF-α, leptin, CRP, adiponectin, and fibrinogen may be biomarkers related to metabolic syndrome.[27]

In another systematic review in which 523 studies were scanned, it has been found that the evidence of 1L1β as a potent biomarker for T2DM was limited.[28]

In Parkinson's disease, the studied parameters in cerebrospinal fluid are thought to be more valuable than the ones studied in serum. In a review published in 2022, it was stated that two inflammatory markers were prominent: Monocyte chemoattractant protein-1 (MCP-1) that is responsible for spreading of inflammation, was shown to be correlated with motor progression. Chitinase-3-like protein 1 (YKL-40) that is expressed by activated microglia was shown to be correlated with faster cognitive decline.[29]

CONCLUSION

In this article, biochemical mechanisms, important mediator molecules, and signal transduction mechanisms in acute and chronic inflammation are summarized in the light of the literature. Although the processes related to acute inflammation have been studied for many years, our knowledge about the processes related to chronic inflammation is more recent. It has been shown that a chronic systemic sterile inflammation plays a role in the pathogenesis of many diseases that affect more than half of the population and in the aging process. Indicators such as leucocyte number, acute phase proteins, hsCRP and MPO can be used in the diagnosis and follow-up of acute inflammation. However, since chronic systemic inflammation can present with very different clinical manifestations, it is difficult to talk about a definite biomarker for its early diagnosis. New information on the chronic systemic inflammation process and signaling mechanisms is being added to the literature every day. In summary, chronic systemic inflammation is associated with especially M1-macrophage polarization, increase of transcription factors (NF-kappaB and AP-1) and an increase accordingly in inflammatory cytokines and nitric oxide. Regarding treatment, studies on inhibition of receptors, signal molecules, and transcription factors involved in signal transmission are carried out intensively. Measures to reduce the causes of chronic systemic inflammation (such as obesity, stress, dietary content, etc.) seem to be the most powerful tools at our disposal for now, since a molecule and/or drug treatment that will act through a single mechanism has not been proven yet.

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in vitro Diagnostics Testing and Future Biochemistry in the Diagnosis of Inflammation

İnflamasyon Tanısında *in vitro* Diyagnostik ve Geleceğin Biyokimyası

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ABSTRACT The study of biochemistry concepts, chemical processes, and *in vitro* diagnostic devices and their related methods is changing very rapidly in the current century. Biochemical diagnostic, measurement of parameters, and verification are very important for the evolving laboratory industry to solve current health problems with new perspectives. Technology and clinical laboratory tests are expeditiously, purposefully evolving. The scientific inquiry has many new routes today and all scientific research areas are trying to find new hypotheses, expectations, theories, formulations, suppositions, assumptions, artificial intelligence, and technology of electronic, electric, and chemical engineering methodologies for a better, healthy, and long life. The scientists are working on developing multifunctional wireless micro-scale miniature robots for diagnostic testing. In near future, the *in vitro* diagnostic tests would be much more accurate, rapid, condition-specific, easy to use, portable, different from the manual and traditional procedures, and performed outside the traditional laboratories with advanced nanorobotic and telecare systems.

Keywords: *in vitro* diagnostics; the imagination of future biochemistry; smart devices; inflammation; micro/nanorobots

ÖZET Biyokimya alanında yeni araştırmalar, kullanılan yeni *in vitro* teşhis cihazları ve bu cihazlar için geliştirilmiş metotlar içinde bulunduğumuz yüzyılda çok hızla değişmektedir. Gelişen biyokimyasal laboratuvar endüstrisinin mevcut sağlık sorunlarını biyokimyasal tanı, parametre ölçümü, doğrulama, yeni bakış açılarıyla çözmesi için çok önemlidir. Teknoloji ve klinik laboratuvar testleri çok hızlı ve amaca yönelik olarak gelişmektedir. Bilimsel sorgulamanın günümüzde birçok yeni rotası vardır. Bütün bilimsel araştırma alanları daha iyi, sağlıklı ve uzun bir yaşam için yapay zeka, elektronik, elektrik, kimya mühendisliği metodolojilerini kullanarak yeni hipotezleri, beklentileri, teorileri, formülasyonları, varsayımları kullanarak çözümlemeye çalışmaktadırlar. Bilim adamları, geleceğin tanı testleri için çok işlevli kablosuz mikro ölçekli minyatür robotlar geliştirmek için çalışıyorlar. Yakın gelecekte *in vitro* diyagnostik tanı testler, önümüzdeki yıllarda çok daha doğru, hızlı, duruma spesifik, kullanımı kolay, taşınabilir, manuel ve geleneksel prosedürlerden çok farklı ve geleneksel laboratuvarın dışında hem evlerde hem de ileri nano robotik ve telebakım sistemler ile gerçekleştirilecektir.

Anahtar Kelimeler: *in vitro* tanı; geleceğin biyokimyasını hayal etmek; akıllı cihazlar; inflamasyon; mikro/nanorobotlar

in vitro diagnostics testing and future biochemistry and managing disease by analyzing body fluids, such as serum, urine, spinal fluid, saliva, and even sweat to investigate the current concentrations of various types of proteins, peptides, enzymes, hormones, minerals, vitamins, immunoglobulins, etc, which are very crucial for determining why the patient is feeling unwell for predictive, preventive and personalized medicine.[1] The digital revolution has transformed many aspects, applications, instruments, methods, and treatments of healthcare systems, industry, and technology. Novel digital technologies are using a combination of several technologies. These novel laboratory technologies are

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begging with knowledge production, laboratory environment, on-site testing for reliability and usability, industry adoption and combination with smartphone applications, telemedicine, symptom reporting applications, computer vision, digital diagnostics and genomics, wearables and sensors, visualization tools, web server, interactive geospatial maps, symptoms reporting applications, drones, data dashboards for efficient to capturing information provide accurate, rapid, case-specific, helpful information for diagnosing, decision making and effective treatment of the disease.[2] The use multidisciplinary approach in laboratory medicine, may be begun with a remote control rollover toy car. These remote-control cars have various properties such as stunt tipping, front and back, right rotation, and stop movements and these properties can be used in *in vitro* diagnostic testing in the future. The idea of the usage of nanorobots in medicine for many scientists may be coming from the 1966 fantastic movie 'Fantastic Voyage'. The imagination was using a submarine-like microrobot to save a patient from a blood clot who is in a coma. However, using nanoparticles in science is much earlier than in this movie. The wireless 3D-printed microrobots are opening new doors in medicine and they have various important properties such as magnetic, biocompatible, soft micro/nanomaterials that can be used in future invasive medicine.[3,4] Researchers have already produced various kinds of micro/nanorobots that can be classified according to their actuation properties such as; magnetic-actuated, biological actuated, acoustics-actuated, and chemical-actuated with different working principles that can operate on tiny areas. These micro/nano devices have different motion forms. The tiny robots can roll, jump, walk, swim, climb and do all these activities that scientists want in a single design. These microrobots can be classified according to their motion forms such as swimming robots, flying robots, and climbing and crawling robots that can easily move inside the human body.[5] The miniaturization of medical robots makes them much more functional in medicine and they become used in the body for therapy, surgery, diagnosis, biopsy, sample collection, tissue penetration, biofilm degradation, biosensor, and medical imaging such as optical imaging, ultrasound imaging, magnetic imaging, radionucleotide imagining and therefore, they must have a special size, function, shape, and material such as combining inorganic elements with biological material.[6] Nature inspired researchers during the evolutionary development of nanomotors and almost all the micro/nanorobots are the micro and nanoscale of most of the living organisms. The nature-inspired nano and microrobots can be classified as; natural morphology inspired, natural structure inspired, natural behavior inspired, and natural behavior and morphology inspired micro and nanorobots.[7] Significant research has been done to develop artificial micro/nanorobots with biocompatible and with natural morphologies such as different plants, animals, microorganisms, and objects used as models. Plants are functionally, anatomically, physically, and structurally very dissimilar then humans and animals, however, trees, coconuts, and walnut were used as models. Plants have micro-nanosystems and their bending-twisting, swelling-shrinking properties, and their shapes and structures were used as a model in 3D and 4D bioprinting, drug delivery, tissue engineering, energy conversion, and harvesting.[7,8] The number, function, and type of animal morphology-inspired micro/nanomotors are more than plant morphology-inspired nanomotors such as fish, jellyfish, tadpole, and annelid worms.[7]

Hybrid micro/nanomotors are offering a novel research area for many scientists and they have various advantages in rapid drug transport, tissue penetration, and motion controllability.[9] Hybrid micro/nanomotors have the advantageous property of cell membrane camouflage.[10] The hybrid micro/nanomotors are designed for the development of biomolecule integrated metal-organic-framework synthetic processes and biomaterials.[11] Red blood cell-based (RBC) micro/nanomotors artificial hybrid nanomotors can combine synthetic nanomotors and natural cell membranes can represent a new class of small motors and can be used in many biomedical and biodefense applications.[7,10] Another type of micro/nanorobots is sperm-based and these micro/nanomotors are used in targeted drug delivery systems the main goal of using these nanorobots is for fighting against cancer. Sperm-based nanomotors have very important swimming performance in complex microenvironments.[7,12] Microorganism-based micro/nanomotors can be seen via microscope because their dimensions are like bacteria (2nm- 300 μm) and they may have flagella.[7]

These structure-inspired micro/nanomotors are the beginning of many novel medicinal applications. In this review, I will discuss the future applications of these micro/nanorobots in possible fields of usage in medicine and *in vitro* diagnostics testing of inflammation shortly.

THE FUTURE OF MICRO/NANOROBOTS AND *in vitro* DIAGNOSTICS

The meaning of *in vitro* is known as in glass from a biochemical point of view we may say as inside the test tube. In the near future, most of the experiments may be run in micro/nanorobots or hybrid micro/nanorobots *in vitro* di-

agnostic tests will no longer be done in a test tube. In this novel technology, enzymes will play a fundamental role in the development of various biochemical tests, which will be done in the new micro/nanorobots. In the future human micro/nanorobots, and implants will be implanted via injection and they will measure not only vital parameters like heart rate, blood pressure, and respiration rate but also all other biochemical tests such as inflammatory parameters.[13] For instance, the level of C-reactive protein (CRP) or high sensitivity C reactive protein (hs-CRP) are very important parameters and it increases when there is inflammation in our body.[14] These micro/nanorobots will measure vital parameters and send a message to our smartphone to take our health care.

Enzymes are accepted as molecular machines and in the future enzyme will be used for enzyme-powered micromotors/nanomotors for converting chemical energy into mechanical motion such as ATPase, urease, catalase, alkaline phosphatase, triose phosphate isomerase, glutathione transferase/urease. They would be used for energy production of these micro/nanorobots and for measuring necessary biochemical analyses for instance blood, and urine parameters. Enzymatic micropumps have great potential for active biosystems, especially in energy production.[15-18] On the other hand stability of enzymes would be affected by environmental conditions such as temperature, pH, and chemicals. Therefore, protection of the enzyme stability and reusability is very important, and this problem can be solved by using enzyme immobilization techniques such as nanoflowers are novel approaches for enzyme immobilization.[19] On the other hand these micro/nanorobots must also contain various types of substrates to measure the specific conditions in the body. Therefore, these required substrates must also be immobilized to measure repetitively and continuously the status of the patient. So, quantum dots are nanometers in size and would be used in the micro/nanorobots for the immobilization of substrates.[20-23]

in vitro DIAGNOSTIC TEST IN MICRO/NANOMOTORS INSTEAD OF TEST TUBES

Test tubes are widely used by biochemistry, microbiology, immunology, and all biomedical science laboratories to handle experiments. The exact design of the test tubes is changing according to the laboratory instruments, diagnostic industry, healthcare systems, assays, and their intended use.[24] In the near future, the scientist and clinical laboratory technicians would not use the standard assay tubes.

There are various types of medical implants made from various materials which are placed inside the body for different medical purposes such as pacemakers and cardioverter defibrillators, devices for insulin administration, deep brain stimulation devices for advanced Parkinson's disease, cochlear implants, contact lens-type ocular *in vivo* dosimeter for radiotherapy.[25-29] Nowadays organ-on-a-chip technology (OOC) is offering superior results in many research areas for instance there are some models for drug toxicity on organs, and it is very crucial to evaluate absorption, distribution, metabolism and excretion, and toxicity assessments. OOC is connecting biochemistry, molecular biology, engineering, and material science research to explain the microenvironment of tissue and organs oxygen concentration gradient, biochemical stimuli, biophysical stimuli, mechanical stimuli, vascularization, immune system components, drug response, the interaction of organs, growth factors, nutrient gradient.[30-32]

The key challenges and gaps that still need to be developed in wearable or embeddable technology in healthcare biosensor applications. Transdermal, skin adherent, optical and mechanical must be evaluating many biochemical and physiological parameters such as the body temperature, predicting the onset of infection and, inflammation sunlight exposure, heart rate, blood pressure, drug level monitoring, insulin, vitamin D, and biotin level, analyzing and calculating motion, walking speed, walking balance and evaluating the kinematics of human body movements. Small biosensors named biostamp were worn by athletes and used for extreme physical conditions and to measure real-time heart rate.[33-36]

The current laboratory tests are done on both normal test tubes and on chips which would be found on wearable biosensors in a very short time, and we would be using micro/nanomotors in our body or under the skin.[37,38]

THE IMAGINATION OF NOVEL SMART DEVICES FOR DIAGNOSTIC TESTS

Smart devices are begging to take place in our homes and the near future we will be talking with these smart devices to plan our daily, weekly, and monthly work in our homes, workplaces, laboratories, and offices for identifying priorities, order tasks, and most of the daily duties. One of the most beautiful smart devices is the toilet which can measure urine volume, flow rate, body mass index, blood glucose, and blood pressure. I hope that the new models of these smart toilets will measure all physical, microscopical, and chemical analyses for instance urine protein, microalbumin, crystals, inflammatory parameters, urinary tract infection, kidney disease, and liver disease, and alert the person who is using it.[39]

Two decades ago small-bowel capsule endoscopy was developed, and automated diagnosis of capsule endoscopy is still developing. Artificial intelligence was added to capsule endoscopy and currently, it gives information about inflammatory bowel disease, cancer, and ulcerative colitis. And in near future, a small laboratory would add capsule endoscopy and they would be much more informative about the status of the patient. They may also take the biopsy from the suspected lesions and they may also be used in targeted therapy.[40]

We need a wide variety of smart devices in our daily life. Perhaps one of the easiest ways to create a smart comb for common problems affecting hair loss, infections, and disorders is because the analysis can be done while combing the hair. Thus, the skin surface microbiome can be easily investigated by smart combs. On the other hand, skin surface and skin diseases are increasing due to the increase in the use of endocrine disruptors in personal care products every year. Hair follicles are affected by foreign molecules and xenobiotics that are foreign to the body, resulting in hair loss. The indicated analysis can be done even in hairdressers so that people's awareness can be increased of the harmful chemicals and endocrine-disrupting chemicals.[41-45] All the personalized products change into smart devices for instance nail files. They can be used by manicurists and/or nail technicians to shape and smooth the nail during manicure and pedicure sessions and at the same time can analyze nail diseases and problems. By using such a smart nail file bacterial and fungal diseases are not contaminated from one person to another.[46,47] Smart toothbrushes would be one of the most important personalized products and they can help to remove plaque from our teeth and prevent periodontal diseases. Most adults who are over 50 years of age may have periodontal diseases and smart toothbrushes may send information to their doctors if there is a problem in tooth health.[48] There is no end to the imagination about the end of *in vitro* diagnostic tests in science and technology. The new area of research would be electronic tattoos. Technology is changing from high-end, high-cost to high-end, low-cost solutions, and with the help of electronic tattoos the *in vitro* diagnostic tests would be evaluated in an extremely different way.[49]

Nanotechnology started at the beginning of the 1980s and due to safety regulations, and hard work in this area every day we are reading a novel nanomedicine, device, or application. There would be a lot of examples of finding the best way of *in vitro* diagnostic tests and nowadays many smart devices communicate in talking technology.[50]

CONCLUSION

in vitro diagnosis is at the core of healthcare and it is always evaluated according to the century's technology. Currently, illness and diseases are evaluated from biological specimens that are taken from the human body but before evaluating these samples the biological samples must be transported to the laboratory. However, in the near future with the help of micro/nanorobots, the biological and physiological status of the patients would be evaluated in high-throughput screening, genomic and cellular analysis done in the body and signals would be sent to the patients, and the doctors' smartphone.

It is very important to integrate the imagination of novel smart devices, technology adaptability, and health care strategy. Scientist is not only creating device, but they are also creating life. The imagination of novel smart devices is the ability, and creativity of combining technology and healthcare. There must be absolute quantification and confidence therefore we need biochemical engineers in the future.

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Classic and New Markers of Inflammation

İnflamasyonda Klasik ve Yeni Belirteçler

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ABSTRACT In response to inflammation, infection, tissue injury and trauma; a complex acute phase response develops in the body. Cytokines such as IL-6, IL-1, TNF-α, IFN-γ and TGF-β are released by macrophages and monocytes, leading to the production or suppression of acute phase proteins by the liver. Substances that increase during inflammation are termed "positive acute phase reactants" (ESR, CRP, SAA, PCT, Ferritin, Fibrinogen, Haptoglobin) and the ones decrease are termed "negative acute phase reactants" (Albumin, Prealbumin, Transferrin, Antithrombin). In recent years, novel inflammatory markers such as Neutrophil to Lymphocyte Ratio, Platelet to Lymphocyte Ratio, Systemic Immune Inflammation Index, Interleukin 6, fecal calprotectin and kynurenine pathway metabolites (quinolinic acid, kynurenic acid, xanthurenic acid) gained importance as sensitive markers of systemic inflammation. Acute inflammatory response can be controlled by appropriate anti-inflammatory treatment and the body can return to the basal homeostatic set points, otherwise ongoing inflammatory state shifts the system to chronic inflammation.

Keywords: Inflammation; acute-phase reaction; biomarkers

ÖZET İnflamasyon, infeksiyon, doku hasarı ve travmaya yanıt olarak vücutta kompleks bir akut faz yanıtı oluşur. Makrofaj ve monositlerden salınan IL-6, IL-1, TNF-α, IFN-γ ve TGF-β gibi sitokinler, karaciğerde akut faz proteinlerinin yapımını artırır ve veya baskılar. Seviyeleri artan bu proteinlere "pozitif akut faz reaktanları" (ESR, CRP, SAA, PCT, Ferritin, Fibrinojen, Haptoglobin), üretimi azalan proteinlere ise "negatif akut faz reaktanları" (Albümin, Prealbümin, Transferrin, Antitrombin) denir. Bunlara ek olarak, kullanımı son yıllarda önem kazanan yeni inflamasyon belirteçleri vardır; başlıcaları Nötrofil/Lenfosit oranı, Platelet/Lenfosit oranı, Sistemik İmmün İnflamasyon İndeksi, İnterlökin 6, kinürenin yolu metabolitleri (kinolinik asit, kinürenik asit, ksantürenik asit) ve fekal kalprotektindir. Akut inflamatuar cevap, uygun anti-inflamatuar tedavi ile kontrol altına alınabilirse sistem normal fizyolojik süreçteki stabil duruma geri döner, inflamatuar yanıt kontrol altına alınamazsa kronik inflamasyon gelişebilir.

Anahtar Kelimeler: İnflamasyon; akut-faz reaksiyonu; biyobelirteçler

Infection and tissue injury are the classic instigators of acute inflammation which trigger the recruitment of leukocytes and plasma proteins to the affected tissue site. The acute inflammatory response is mediated by tissue resident macrophages and mast cells, leading to the production of a variety of inflammatory mediators, including chemokines, cytokines, vasoactive amines and eicosanoids.

Inflammation can be induced by exogenous or endogenous agents. Exogenous inducers can be classified into two groups as microbial and non-microbial factors. Microbial factors are bacterial and viral infections. Non-microbial factors include allergens, irritants and toxic compounds silica and asbestos. Endogenous inducers of inflammation are signals produced by stressed, damaged or malfunctioning tissues which cause chronic inflammation. Autoimmune diseases, type 2 diabetes and cardiovascular diseases are associated with chronic inflammation.[1]

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Regardless of the cause, the inflammatory response try to remove or sequester the source of the disturbance, to allow the host to adapt to the abnormal conditions and, ultimately, to restore functionality and homeostasis to the tissue. If the abnormal conditions are transient, then a successful acute inflammatory response returns the system to the basal homeostatic set points. If, by contrast, the abnormal conditions are sustained, then an ongoing inflammatory state shifts the system to different set points, as occurs during chronic inflammation.

In response to injury, local inflammatory cells (neutrophil, granulocytes and macrophages) secrete a number of cytokines into the bloodstream, such as interleukins IL1, IL6, IL8, and TNFα. The liver responds by producing a large number of plasma proteins that increase or decrease in response to inflammatory stimuli. "Positive acute-phase reactants" are the plasma proteins whose concentration is significantly increased in the acute inflammatory processes. Strong acute phase proteins responds rapidly to inflammatory stimuli and their serum levels may increase several hundred folds, moderate acute phase proteins can increase only 2-10 folds. At the same time, the production of a number of other proteins is reduced, these are referred as "negative acute-phase reactants".[2] Classic and new markers of inflammation are given in Table 1.

POSITIVE ACUTE PHASE REACTANTS

ERYTHROCYTE SEDIMENTATION RATE

Erythrocyte sedimentation rate (ESR) is a non-protein indirect acute phase reactant which is used to monitor the inflammatory activity caused by autoimmune diseases, infections or tumors. ESR measures the distance at which the red blood cells in anticoagulated whole blood has fallen down in one hour due to the influence of gravity. The Westergren method was adopted as the gold standard for ESR measurement in1973. Although automated machines were developed for the analysis of the ESR, the Westergren method was still confirmed as the gold standard in 2011 by both the International Committee for Standardization in Haematology (ICSH) and by the Clinical and Laboratory Standards Institute (CLSI).[3]

The ESR determines the presence of inflammatory activity caused by autoimmune diseases, infections or tumors. It begins to rise within 24-48 hours of the onset of inflammation, decreases slowly as inflammation resolves, and can take weeks to completely normalize, usually used in combination with other tests. Normal ESR value is higher in females than in males and increased gradually with age; generally calculated in men as age/2 and in women as (age +10)/2. ESR in men younger than 50 years old is ≤15 mm/h, whereas in female younger than 50 years old is ≤20 mm/h. In male older than 50 years old ESR is ≤20 mm/h and female older than 50 years old ESR is ≤30 mm/h. Importantly, investigation of underlying etiology should be carried out in patients with ESR values greater than 100 mm/h. High ESR value (>100 mm/h) may indicate the presence of infection, multiple myeloma, Waldenstrom macroglobulinemia, temporal arteritis, polymyalgia rheumatica or hypersensitivity vasculitis.[4] The factors affecting ESR are given in Table 2.

TABLE 1: Classic and new markers of inflammation.

Classic Markers of Inflammation		**New Markers of Inflammation**
Positive Acute Phase Reactants	**Negative Acute Phase Reactants**	Cellular Inflammation Markers
Strong APP	Albumin	- NLR
ESR	Prealbumin	- PLR
CRP	Transferrin	- SII
SAA	Antithrombin	- IL-6
PCT		Kynunerine Pathway Metabolites
Moderate APP		- Kynurenine (Kyn)
Ferritin		- Tryptophan (Trp)
Fibrinogen		- Kyn/Trp ratio
Haptoglobin		Calprotectin

APP: Acute Phase Protein, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, SAA: Serum Amyloid A, PCT: Procalsitonin, NLR: Neutrophil/ Lymphocyte Ratio, PLR: Platelet/ Lymphocyte Ratio, SII: Systemic Inflammation Index, IL-6: Interleukin-6.

TABLE 2: Factors affecting ESR.

Factors Increasing ESR	Factors Decreasing ESR
Diseases: Anemia, Hypercholesterolemia, Obesity, Pregnancy, Infections, Chronic Kidney Disease, Menstruration, Malignancies, Myeloma, Inflammatory Bowel Diseases, Collagen Tissue Diseases, Amyloidosis, Autoimmune Diseases, Hypothyroidism, Diabetes Mellitus, Burn, Tissue Damage (AMI).	**Diseases:** Erythrocyte Anomalies, Sickle Cell Anemia, Spherocytosis, Anisocytosis, Microcytosis, High Leukocyte Count, Excessive Anticogulation, Polycythemia, Hypofibrinogenemia (DIC, Massive Hepatic Necrosis), Liver Failure, Congestive Heart Failure, Cachexia and Anorexia.
Drugs: Dextran, Theophylline, Methyl dopa, Methylcergitis, Penicillamine, Vitamin A, Trifluperidol, Oral Contraceptives.	**Drugs:** Quinine, Salicylates, Valproic Acid, High Dose Steroids, Low Molecular Weight Dextran.
Technical Reasons: Shaking of the sedimentation tube, high room temperature, exposure to direct sunlight.	**Technical Reasons:** Low room temperature, coagulation of the sample due to long preanalytical phase (more than 2 hours).

ESR: Erythrocyte sedimentation rate, AMI: Acute Myocard Infarctus, DIC: Dissemine Intravascular Coagulation.

C-REACTIVE PROTEIN

C-Reactive Protein (CRP) is an important regulator and marker of inflammatory processes; exhibits elevated expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases and infection. CRP is synthesized primarily by hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes. CRP was discovered by Tillet and Francis in 1930 in patients with pneumococcal pneumonia, where it was found to interact with the C-polysaccharide of *Streptococcus pneumoniae* cell wall, hence the term C-reactive protein.[5]

CRP is produced as a homopentameric protein, termed as pentameric CRP (pCRP), which can irreversibly dissociate at sites of inflammation and infection into five separate monomers, termed monomeric CRP (mCRP) as shown in Figure 1. These five identical monomers are noncovalently bound to each other, each 206 amino acids long with a molecular mass of about 23 kDa. The mCRP molecules are distinguished from pCRP by their different antigenic and biological characteristics: pCRP isoform activates the classical complement pathway, induces phagocytosis, and promotes apoptosis whereas mCRP promotes the chemotaxis and recruitment of circulating leukocytes to areas of inflammation and can delay apoptosis. mCRP increases IL-8 and MCP-1 production, whereas pCRP has no detectable effect on their levels. Mild increases in CRP between 2 mg/L and 10 mg/L are considered to be metabolic inflammation. In acute inflammatory conditions, CRP can rise as much as 50-100 mg/L within 4-6 hours of a mild to moderately noxious stimulus, such as an uncomplicated skin infection, cystitis or bronchitis. Elevated levels of CRP (>100-500 mg/L) are strongly associated with bacterial infections. CRP levels double every 8 hours and peak 36-50 hours after the onset of inflammation or injury. However, when the stimuli ends, CRP values decrease exponentially over 18-20 h, close to the half-life of CRP.[6]

There are many factors that can alter baseline CRP levels including age, gender, smoking status, weight, lipid levels and blood pressure. Oral hormone replacement therapy (HRT) increases CRP levels in postmenopausal women, increasing the risk of thrombosis whereas transdermal estrogen supplementation has little effect on circulating CRP levels.[7]

CRP usually correlates with the erythrocyte sedimentation rate (ESR), however not always directly. This is due to the ESR being largely dependent on elevation of fibrinogen, has a much longer half-life than CRP, making ESR helpful in monitoring chronic inflammatory conditions, whereas CRP is more useful in diagnosis as well as in monitoring responses to therapy in acute inflammatory conditions, such as acute infections.[8]

SERUM AMYLOID A

Acute-phase reactant serum amyloid A (A-SAA) plays an important role in acute and chronic inflammation, used as an indicator of inflammatory processes and monitoring the efficacy of medical therapy. SAA has greater clinical diagnostic value than CRP in patients with viral infections, severe acute pancreatitis, rejection of kidney transplants, sarcoidosis, patients using immunosuppressive drugs (cyclosporine and corticosteroids) and preterm infants with late-onset sepsis. Compared with CRP levels, A-SAA levels have been reported to increase faster and reach higher levels. In healthy individuals, SAA levels is 20-50

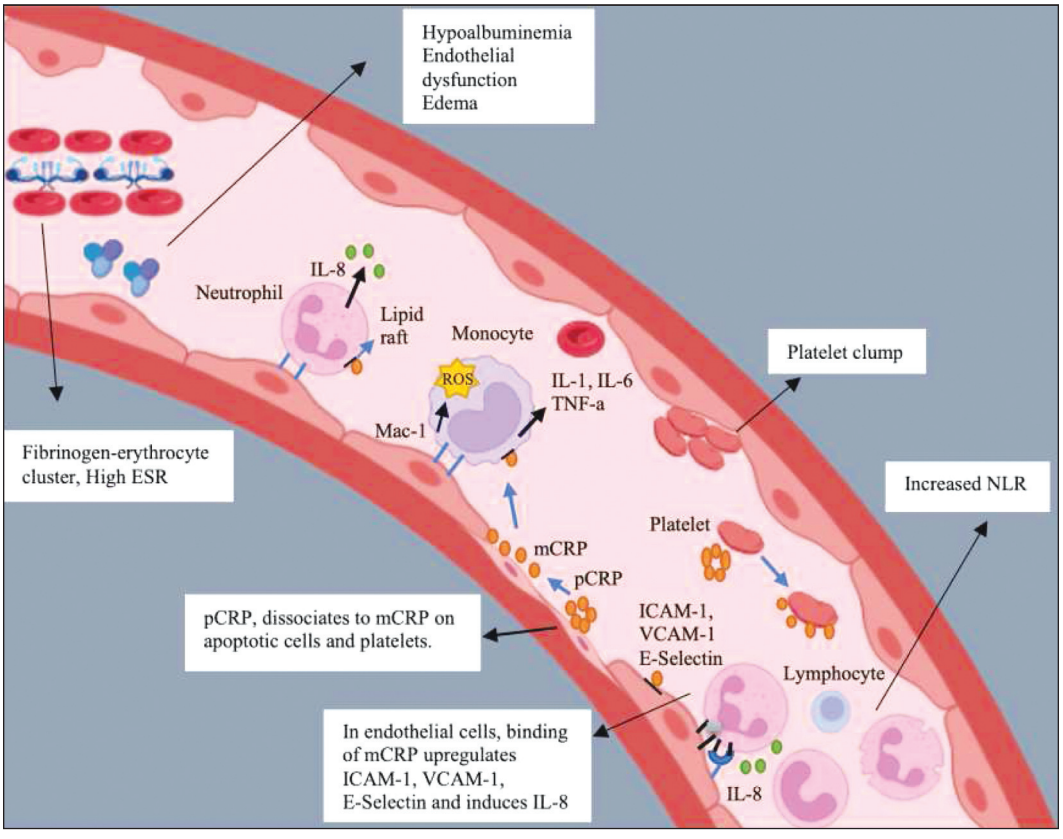


FIGURE 1: Hallmarks of inflammatory response.

ESR: Erythrocyte Sedimentation Rate, pCRP: Pentameric C-Reactive Protein, mCRP: Monomeric C-Reactive Protein, ICAM-1: Intercellular Adhesion Molecule 1, VCAM-1: Vascular Cell Adhesion Molecule 1, IL: Interleukin, TNF-a: Tumor Necrosis Factor-a, NLR: Neutrophil/ Lymphocyte Ratio.

mcg/mL. However, their levels can rise as much as 1000-fold following 24-36 hours after onset of inflammation. After the resolution of the inflammation, SAA may return to basal level more quickly than CRP due to its shorter half-life. Furthermore, the currently available SAA assays are based on polyclonal anti-bodies, which lack isotype specificity and are associated with many inflammatory diseases. Therefore, the combinatorial use of SAA, with CRP and procalcitonin can provide more information for the assessment of the inflammation status.[9]

Serum amyloid A is a precursor of protein amyloid A in secondary amyloidosis deposited in interstitium of tissues and interfere with normal tissue function. Serum amyloid A comprises multiple isoform designated SAA1, SAA2 and SAA4. The isoforms SAA1 and SAA2 are up-regulated by inflammatory cytokines.[10]

PROCALCITONIN (PCT)

PCT is a 116 amino acid peptide precursor of calcitonin, produced in the C-cells of the thyroid gland and increases within 4-12 h in the presence of bacteria, exhibiting a half-life of 24 hours. The release of PCT is enhanced by tumor necrosis factor, interleukin-1 and interleukin-6, typically seen in bacterial infections, but inhibited by virus-induced interferon-γ during viral infections. PCT has been lauded as a useful early diagnostic biomarker of septic events especially differentiating bacterial and non-bacterial etiologies, predict mortality and as a guide for the management of antibiotic therapy in critically ill patients. PCT appears to be particularly sensitive to bacterial toxins compared to other biomarkers such as white blood cells and CRP, and it undergoes down-regulation in the presence of viral infections. PCT levels are lower than 0.1 μg/L in healthy people. In the presence of infection, PCT levels are 0.5-2 μg/L, most studies agreed to begin the antimicrobial therapy when PCT >0.5 ng/mL. PCT levels between 2-10 μg/L predicts sepsis whereas PCT>10 μg/L predicts strong sepsis.[11-13]

PCT detects bacterial infections with 79% specificity; higher PCT levels is observed in Gram-negative bacteremia than in Gram-positive bacteremia and fungal sep-

sis. PCT level decreases by 50% every 24 h with appropriate antibiotic treatment. In 2017, PCT was approved as a marker to guide antibiotic therapy in lower respiratory tract infections, by the US Food and Drug Administration. The therapy should be terminated when PCT <0.5 μg/L or when there is an 80% reduction from the initial value.[13]

FERRITIN

Serum ferritin is used as a marker of iron deficiency but it is also an inflammatory biomarker and well-known acute-phase reactant. The normal range of serum ferritin is 18–350 ng/mL. Increased ferritin levels can be observed at acute and chronic inflammation in bacterial and viral infectious, rheumatologic, hematologic and malignant disease. Hyperferritinemia, is associated with high mortality; patients show a typical pattern of reticuloendothelial system activation and multiple organ dysfunction.[14] The failure to control iron homeostasis can lead to iron-associated neurodegenerative disorders. The levels of serum ferritin are significantly higher in patients with ALS, Parkinson's disease and Alzheimer's Disease, compared with healthy subjects.[15]

Serum ferritin serves as a marker of cellular damage as it arises from damaged cells. In inflammatory conditions, ferritin loose its iron, the free unliganded iron catalyses hydroxyl radical formation which lead to oxidative damage. The red blood cells loose their typical discoid shape and their elastic ability while the fibrin network forms a dense matted layer. RBCs are entrapped in the pathological rigid fibrin clot that resistant to fibrinolysis.[16]

In recent years the interrelationship between ferritin, glucose metabolism, arterial stiffness and inflammation has been extensively investigated. Scientific evidence has shown that ferritin levels have been associated new-onset Type II DM risk and increased arterial stiffness, atherosclerotic injury and vascular damage.[17]

FIBRINOGEN

Fibrinogen is a soluble symmetrical 340-kDa glycoprotein synthesized in the liver composed of three different polypeptide chains (Aα, Bβ, and γ). These chains make an elongated molecule which binds to a second identical molecule by disulfide bonds, forming the homodimeric fibrinogen molecule. Under physiological conditions, plasma concentrations of fibrinogen range from 2-4 g/L, and fibrinogen has a half-life of approximately 4 days. However, in pathological conditions, such as after injury, or disease associated with vascular disruption, infection, or inflammation, the blood concentration of fibrinogen increases several fold. Fibrinogen acts on different cell types (monocytes, mast cells, platelets, macrophages, microglia, endothelial cells) through cell-specific integrin and non-integrin receptors to induce specific inflammatory functions. Proinflammatory role for fibrinogen has been reported in vascular wall disease, stroke, spinal cord injury, brain trauma, multiple sclerosis, Alzheimer's disease, rheumatoid arthritis, bacterial infection, colitis, lung and kidney fibrosis, Duchenne muscular dystrophy, and several types of cancer.[18]

Some physiological factors and life style can influence fibrinogen levels. Increasing age and female sex, winter season, pregnancy and oral contraception, menopause, smoking and exercise can increase fibrinogen. Reduced fibrinogen levels can be observed at viral hepatitis, decompensated liver disease and DIC. Elevation in fibrinogen level increase plasma viscosity, binding of fibrinogen to activated platelets via glycoprotein IIb/IIIa, promotes platelet aggregation and fibrin formation. Hence, high plasma fibrinogen levels are associated with increased risk of thromboembolism and cardiovascular diseases.[19]

RBCs are negatively charged and tend to repel one another. The presence of positively charged, large, asymmetric acute phase proteins such as fibrinogen and immunoglobulins cause an increase in ESR. Due to the long half-life of fibrinogen, the ESR does not change rapidly at the beginning of the inflammatory process and normalizes more slowly than that of other acute phase reactants as shown in Figure 1.

HAPTOGLOBIN

Haptoglobin is an acute phase protein that scavenges haemoglobin in the event of intravascular or extravascular haemolysis. It is an antioxidant protein produced by liver. Haptoglobin levels decrease during malnutrition, hemolysis, hepatic disease, allergic reactions, and seizure disorders. Parasitic (malaria), infectious (HIV, tuberculosis) and non-infectious diseases (diabetes, cardiovascular disease and obesity), inflammation, and malignancies cause increase in haptoglobin levels. The protein exists in humans as three main phenotypes, as Hp1-1, Hp2-2 and Hp2-1. Phenotype-dependent poor disease outcomes have been linked with the Hp2-2 phenotype.[19]

NEGATIVE ACUTE PHASE REACTANTS

ALBUMIN

Albumin is a negative acute phase reactant which makes up 60% of serum proteins. Albumin plays a regulatory role in acid-base physiology, binding of essential components

in blood circulation and transport to organs, inhibition of platelet function, vascular permeability, protection of colloid-osmotic pressure, and free radical capture, antioxidant activities are also available. Hepatocytes produce 9-12 g of albumin daily, with a half-life of 19 days. Albumin has a half-life of 20 days, albumin levels return to normal within 2 weeks when the body pool has been depleted. Hypoalbuminemia can be seen in acute and chronic inflammatory conditions. Albumin levels are often used by clinicians to assess the nutritional status of patients. Hypoalbuminemia (<3.5 g/dL) can develop due to chronic liver diseases, chronic kidney disease, inflammation, malnutrition, infections, cancer, extensive burns, pregnancy, shock, sex hormones, insulin and steroids. Hypoalbuminemia occurs in inflammatory conditions due to inflammatory cytokines (TNF-α, IL-1, IL-6) which suppress albumin production and increase fractional catabolic rate.[20]

Inflammation also leads to increased permeability of the vascular endothelial cells and escape of albumin out of the vascular compartment, leading to hypoalbuminemia as shown in Figure 1. Each albumin has the capacity to hold 18 ml of fluid in the intravascular space. Albumin loss cause accumulation of fluid in the extravascular space that leads to peripheral edema in the extremities and pulmonary edema. ARDS may also develop as a result of alveolar epithelial damage and capillary escape.

Several biochemical parameters can be affected due to hypoalbuminemia. In hypoalbuminemic states, the level of ionized free Ca and indirect bilirubin increases, the transport of drugs is disturbed, and hyperlipidemia developes. Consequently, hypoalbuminemia prolongs the hospitalization process and increases the risk of developing complications. The goal of the treatment is to eliminate the inflammatory factor that causes hypoalbuminemia followed by albumin replacement.[21]

FIBRINOGEN/ALBUMIN RATIO

Fibrinogen to albumin ratio (FAR) has been reported as a new marker of inflammation. It has been observed that the ratio of these two parameters is closely related to many inflammation based diseases such as coronary artery diseases, diabetic kidney diseases and spondyloarthritis.[22-24]

CRP/ALBUMIN RATIO

CRP/Albumin ratio (CAR) is an inflammatory marker that is considered to have prognostic value in various cancers, acute pancreatitis, ulcerative colitis and sepsis. CRP/albumin levels at ICU (intensive care unit) correlate with long-term prognosis at 90 days in septic patients. CRP/albumin ratio >2 showed the greatest sensitivity and specificity in predicting mortality at 90 days. As a long-term marker of prognosis, CRP/albumin ratio provides more consistent data than CRP values alone.[25]

PREALBUMIN (TRANSTHYRETIN)

Prealbumin is a negative acute phase reactant located in front of albumin during the electrophoretic migration. Prealbumin is primarily synthesized in the liver, it is a transport protein for thyroxine and retinol with a half-life of 2 days, eliminated by the kidneys and gastrointestinal tract. It is used both for the diagnosis of protein energy malnutrition and in the follow-up of treatment, especially both in the geriatric patient population. Compared to albumin, prealbumin has a more rapid rate of hepatic synthesis, but a shorter half-life and higher catabolic rate. Therefore, prealbumin is a more potentially sensitive marker to assess nutritional status and for short-term outcome compared with albumin. Although malnutrition is one of the most common geriatric syndromes in the elderly, it is also a determinant of morbidity and mortality. Prealbumin is associated with health conditions such as increased length of stay in hospital, decreased quality of life, delayed wound healing, infection and decreased functional capacity. Low levels may directly activate osteoclasts and inhibit osteogenesis via NF-κB; therefore increase the risk of osteoporosis-related fractures and post-fracture healing. Reduced prealbumin is associated with bone mineral density in postmenopausal women with osteoporosis.[26]

The normal reference range for prealbumin is 15-36 mg/dL, but changes with age and gender. The range for the geriatric population is lower than the reference range for the adult age group, this condition is thought to be related with decreased IGF-1 by aging. Prealbumin synthesis is controlled by sex steroid hormones, hence the reference range values for women are slightly lower than those for men. Prealbumin levels decrease due to insufficient calorie intake, liver dysfunction, infection, hyperthyroidism, dialysis and over hydration. Prealbumin levels may increase due to severe renal failure, non-steroidal anti-inflammatory agents, corticosteroids, oral contraceptives and dehydration.[27] Pro-inflammatory cytokines (IL-1, IL-6, TNF-α) secreted in inflammatory conditions reduce the production of prealbumin. In Acute Kidney Injury (AKI) patients, inflammation and protein-energy wasting are common and strongly associated with mortality. Low levels of serum prealbumin (<15 mg/dL) in AKI patients has been shown to correlate with the poor survival up to 90 days.[28]

TRANSFERRIN

Transferrin is a 76 kDa glycoprotein capable of reversible iron binding, produced mainly in the liver, smaller amounts in brain, lymph nodes, testicular tissue and mammary glands. It is a negative acute phase reactant with a half-life about 8 days in the serum. Serum transferrin levels is approximately 200-300 mg/dL but reduced in inflammatory conditions, malnutrition and anemia.[29]

Transferrin mediates iron exchange between iron from donors as enterocytes and macrophages, to acceptor cells like erythroblasts. By sequestering iron, transferrin prevents its involvement in free radical production by Haber-Weiss reaction. Hence, it has an important antioxidant capacity was associated with the activities of enzymatic antioxidant enzymes such as paraoxonase-1, erythrocyte superoxide dismutase-1, catalase and glutathione peroxidase. Inflammation triggers anemia of chronic disease as proinflammatory cytokines interfere with iron homeostasis. Transferrin is an iron deficiency marker, superior to saturation-based indices and iron levels Transferrin contributes to malnutrition by affecting muscle protein turnover, is also considered to be a reliable measure of overall protein status, it positively correlates with albumin.[30]

ANTITHROMBIN

Antithrombin is a natural anticoagulant produced in the liver. Although it is one of the major endogenous anticoagulants in the body, it is also a negative acute phase reactant. They interact with heparan sulfate proteoglycans located on the surface of cells and active proteases of the coagulation system. Antithrombin levels decrease in inflammatory conditions. The reason for this decrease is its consumption by the coagulation system and immunological processes.[31]

NEW MARKERS OF INFLAMMATION

CELLULAR IMMUNE INFLAMMATION MARKERS

Although total leukocyte count is a useful measure to depict infammation, cellular immune inflammation markers such as Neutrophil to Lymphocyte Ratio (NLR), Platelet to Lymphocyte Ratio (PLR) and Systemic Immune Inflammation Index (SII) provide a multifactorial insight into inflammatory processes. NLR is a new addition to inflammatory markers (Figure 1). NLR, which is calculated from complete blood count, hence, is an inexpensive available marker of inflammation. NLR has been associated with arterial stiffness and high coronary calcium score, therefore NLR is reported as an independent predictor of outcome in stable coronary artery disease, as well as a predictor of short- and long-term mortality in patients with acute coronary syndromes.[32]

A second cellular immune inflammation marker is the PLR. Beside the well-known role of platelets in primary haemostasis, they also exhibit various pro-inflammatory properties, underlining their value as inflammation marker. High PLR and elevated platelet count are useful for the diagnosis of systemic vasculitides.[33] PLR and NLR have high predictive value in rheumatic diseases with predominantly neutrophilic inflammation such as Behçet's disease and Familial Mediterranean fever.

SII is as a third cellular immune inflammation based prognostic marker that integrates the kinetics of NLR and PLR into one single parameter. SII has been recently suggested to be associated with poor outcome of cancer, and a significant correlation between SII and number of circulating tumor cells have been repoted.[34]

Multi-Center Study from Western China was done to determine the normal reference intervals of NLR, PLR and SII in healthy adults (n=5,969). They found that the gender can significantly influence PLR and SII ($p<0.05$), but not NLR ($p>0.05$). Surprisingly, increase in age caused a decrease in PLR, and SII while NLR remained stable. PLR and SII values were significantly higher in the young adults (18-64 years) compared to old adults (65-79 years) ($p<0.001$). The reference intervals of NLR, was 0.88-4.0, PLR (young adults), 49-198, PLR (old adults), 42-187, and SII were 142-804 x 10^9/L, respectively.[35]

INTERLEUKIN 6 (IL-6)

Human IL-6 is made up of 212 amino acids, including a 28-amino-acid signal peptide, and its gene has been mapped to chromosome 7p21. Although the core protein is ~20 kDa, glycosylation accounts for the size of 21-26 kDa of natural IL-6. Under healthy conditions the serum level of IL-6 is less than 4 pg/mL, but when stresses such as infections or tissue injuries occur, IL-6 is immediately produced by monocytes and macrophages in response to infections and tissue injuries. After IL-6 is synthesized in a local lesion in the initial stage of inflammation, it moves to the liver through the bloodstream, followed by the rapid induction of an extensive range of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, haptoglobin and α1-antichymotrypsin, in contrast decreases the production of fibronectin, albumin and transferrin. IL-6 plays a key role in cytokine storm with a pleiotropic effect on inflammation, immune response and hematopoiesis by activating both complement components and coagulation pathway.

Once stress is removed from the host, IL-6 synthesis ends, but uncontrolled excessive or persistent IL-6 production plays a pathological role in the development of various chronic inflammatory diseases and cancers. IL-6 acts as a paracrine or autocrine growth factor for many cancer cells and induces cancer-related inflammation. It is associated with worsening of the prognosis for cancer by promoting angiogenesis, metastasis and modification of immunity. Remarkable beneficial effects of IL-6 blockade therapy using a humanized anti-IL-6 receptor antibody, tocilizumab were recently observed in patients.[36,37]

KYNURENINE PATHWAY METABOLITES

Tryptophan (Trp) is the least abundant essential amino acid used in protein synthesis. Tryptophan is metabolized by the liver to maintain serum concentrations of 50-100 μM. The main catabolic pathway of tryptophan in mammals is the pathway of kynurenine. At the end of this pathway, nicotinamide adenine dinucleotide (NAD^+) synthesis takes place. This pathway is responsible for >90% of peripheral Trp metabolism in mammals.[38] Trp is predominantly converted into kynurenine (KYN) by the indoleamine 2,3-dioxygenase (IDO) isozymes and tryptophan 2,3-dioxygenase (TDO). IDO-1 is expressed by various immune cells in the body such as dendritic cells, monocytes and macrophages. It is metabolized to kynurenic acid (KYNA) by the kynurenine aminotransferase (KAT) isozymes of kynurenine. KYNA is thought to be neuroprotective. In addition, it can be metabolized to 3-hydroxykynurenine (3HK) by kynurenine mono-oxygenase (KMO) and to anthranilic acid by kynureninase. Metabolites in this pathway are generally considered to be neurotoxic. For example, 3HK released in this pathway is a free radical generator, while quinolinic acid (QA) released towards the end of this pathway is a neurotoxin and gliotoxin. QA is the endogenous source of nicotinamide and nicotinamide adenine dinucleotide (NAD^+).[39] In cases of inflammation, proinflammatory cytokines including interferon gamma and TNF-α signifi-

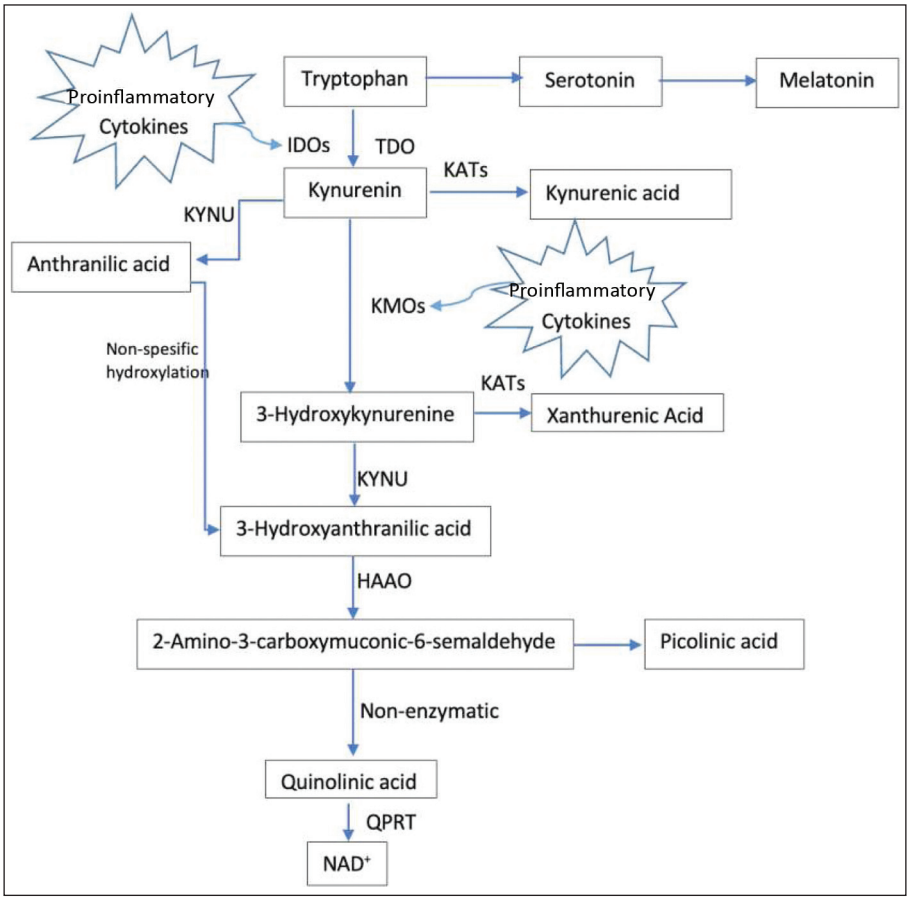


FIGURE 2: Kynurenine pathway and metabolites.

IDOs: Indoleamine 2, 3-dioxygenase izozymes, TDO: Tryptophan 2,3-dioxygenase, KATs: Kynurenine aminotransferase, KMOs: Kynurenine mono oxygenase, KYNU: Kynureninase, HAAO: 3 hydroxyanthranilate 3,4-dioxygenase, QPRT: Quinolinic acid phosphoribosyltransferase, NAD: Nicotinamide Adenine Dinucleotide.

cantly upregulate the indoleamine 2,3-dioxygenase involved in the first step of the Kynurenine Pathway (KP). Inflammation mediated upregulation of this enzyme results in increased catabolic turnover of tryptophan leading to accumulation of KP metabolites, which simultaneously reduces the amount of tryptophan available for the synthesis of serotonin.[40] IDO is activated primarily via the interferon gamma receptor (IFN-γR). The increase in CNS (Central Nerve System) concentrations of KYN occurs through increased transport of KYN to the brain during systemic inflammation, by an IDO-independent mechanism. QA is required for sufficient NAD production. NAD is very important in terms of the energy that the activated immune cells need to cope with the infection. For this reason, in inflammatory conditions, an increase in metabolism can be seen in the QA branch of KP.[39] KMO but not KAT-II expression is increased in human hippocampal progenitor cells, IL-1β was shown to increase KMO transcripts.[41] Cholesterol lowering treatment in CKD (Chronic Kidney Disease) patients during 1 year has significantly decreased both KYN and KYN/TRP ratio compared to healthy controls.[42] As a result, metabolites of the kynurenine pathway increase, tryptophan decreases, and the KYN/TRP ratio increases in cases of inflammation. Activation of the kynurenine pathway reduces serotonin production from tryptophan. Low serotonin levels lead to depression and reduce the activity of pain relief pathways. The most frequently detected biomarker in patients with chronic pain is high Quinolinic acid level (29%). Kynurenine pathway is also associated with depression and chronic pain conditions, especially inflammation.[40]

CALPROTECTIN

Faecal calprotectin is a calcium-binding protein secreted into the intestinal mucosa by the white blood cells, neutrophils, monocytes and macrophages in the inflammed areas of the bowel. Calprotectin remains stable against bacterial degradation during intestinal transit. It is a non-invasive marker of gastrointestinal inflammation, more sensitive than systemic inflammatory markers for differentiating inflammatory bowel disease (Crohn's disease and Ulcerative colitis) and non-inflammatory bowel diseases (irritable bowel syndrome).[43] It is recommended that stool sample should be collected from the first bowel action of the day, and kept for no longer than 3 days at room temperature. The cut-off value for faecal calprotectin is 50 μg/g, levels between 50-100 μg/g is accepted as borderline elevations; mildly elevated levels (100-200 μg/g) usually requires more extensive investigation.[44]

Calprotectin levels correlate with the clinical disease activity; increased faecal calprotectin (>100 μg/g) indicates the relapse, usually rise occurs median of 94 (13-317) days before clinical symptoms, which helps early start of treatment. Decrease in faecal calprotectin is associated with mucosal healing and clinical remission, hence can be used for decision-making about stopping therapy. Faecal calprotectin is not specific for inflammatory bowel disease and can also be increased in infectious enteritis, polyps, diverticulitis, in patients using NSAID and colorectal neoplasia. Hence, faecal calprotectin test is not appropriate in patients older than 50 years, as colonic polyps and malignancy are more common.[45]

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Clinical Applications of Cytokines as Inflammation Markers: An Overview

İnflamasyon Belirteci Olarak Sitokinlerden Klinik Yararlanım: Genel Bakış

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ABSTRACT Inflammation plays a pivotal role in various diseases, and the identification of reliable biomarkers is crucial for diagnostic and therapeutic advancements. Cytokines, as signaling molecules, including interleukins, interferons, tumor necrosis factors, and chemokines, have a variety of pro-inflammatory and anti-inflammatory effects on the body through different biochemical pathways and interactions. These critical mediators, which monitor and regulate immune and inflammatory responses through complex networks, serve as biomarkers for many diseases. Stimuli, actions, interactions, and downstream effects of cytokines have been studied more extensively in recent years, and clinical research has also sought to incorporate cytokines into causal patterns to aid diagnosis and treatment. This review provides an overview of the evolving landscape of cytokines as markers of inflammation and offers insights into their diagnostic precision, prognostic significance, and therapeutic potential in various inflammatory diseases.

Keywords: Cytokine; inflammation; biomarker

ÖZET İnflamasyon, çeşitli hastalıklarda belirleyici bir rol oynar, tanı ve tedavideki gelişmeler bakımından güvenilir biyobelirteçlerin tanımlanması çok önemlidir. İnterlökinler, interferonlar, tümör nekrozis faktörler ve kemokinleri içeren ve sinyal molekülleri olan sitokinler vücutta farklı biyokimyasal yolaklar ve etkileşimler üzerinden çeşitli pro-inflamatuar ve anti-inflamatuar etkiler gösterirler. Bu kritik medyatörler karmaşık bir iletişim ağı içinde immün ve inflamatuar yanıtları izleyerek ve düzenleyici rol üstlenerek birçok hastalık için biyobelirteç görevi yaparlar. Sitokinlerin uyarıcı, etkileyici, etkileşimci ve akış yönündeki görevleri son yıllarda geniş kapsamlı çalışılmaktadır. Klinik çalışmalarda tanı ve tedaviye yardımcı olarak sitokinlerin rol aldığı nedensel modeller üzerinde araştırmalar yapılmaktadır. Bu derleme, inflamasyon belirteçleri olarak sitokinlerin gelişen durumuna genel bir bakış sağlamakta ve çeşitli inflamatuar hastalıklarda bunların tanısal kesinliği, prognostik önemi ve terapötik potansiyeli hakkında bilgiler sunmaktadır.

Anahtar Kelimeler: Sitokin; inflamasyon; biyobelirteç

Inflammation is a complex biological response that plays a pivotal role in health and disease. It is associated with a wide range of conditions, including autoimmune disorders, infectious diseases, and cancer. The identification and monitoring of inflammation markers are of paramount importance for early diagnosis, disease monitoring, and the development of targeted therapies. Cytokines, as key regulators of the immune response, have garnered significant attention as potential biomarkers for inflammation. This review aims to provide an overview of the current state of knowledge on the clinical utilization of cytokines as inflammation markers, with a focus on their diagnostic, prognostic, and therapeutic applications.

CYTOKINES: KEY PLAYERS IN INFLAMMATION

DEFINITION AND CLASSIFICATION

Cytokines are a diverse group of small proteins, peptides, or glycoproteins that are secreted by specific cells of the immune system. Cytokines are a category of signaling

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molecules that serve as intercellular messengers in the immune system and regulate immunity, inflammation, and hematopoiesis. A large family of cytokines is produced throughout the body by cells of various embryologic origins. Cytokine is a generic name; other names are defined based on their presumed function, the cell from which they are secreted, or the target of their action. For example, cytokines produced by lymphocytes may also be called lymphokines. Many of the lymphokines are also referred to as interleukins (ILs) because they are not only secreted by leukocytes but can also influence the cellular responses of leukocytes. The cytokines secreted by monocytes or macrophages are called monokines. Chemokines are cytokines with chemotactic activities. The cytokine superfamily can be roughly divided into different categories, including interleukins, chemokines, colony-stimulating factors (CSF), interferons, and the families of transforming growth factors (TGF) and tumor necrosis factors (TNF). The number of human cytokines and chemokines identified to date is two hundred and thirty-one.[1,2]

Cytokines are produced by many cell populations, but the most important producers are T helper cells (Th) and macrophages. Cytokines can be produced in and by peripheral nerve tissue during physiologic and pathologic processes by resident and recruited macrophages, mast cells, endothelial cells, and Schwann cells. Following peripheral nerve injury, macrophages and Schwann cells that congregate around the injured area of the nerve secrete cytokines and specific growth factors that are necessary for nerve regeneration. Local inflammatory irritation of the dorsal root ganglion (DRG) leads not only to an increase in pro-inflammatory cytokines but also to a decrease in anti-inflammatory cytokines. Cytokines can also be synthesized and released from the herniated nucleus pulposus, which is synthesized in the spinal cord, DRG soma, or inflamed skin. In addition, cytokines can be transported retrogradely from the periphery via axonal or non-axonal mechanisms into the DRG and dorsal horn, where they may have a beneficial effect.[3]

Cytokines exist in broad families that are structurally related but can have quite different cytokine functions. The properties of the cytokines are defined in Table 1. The cytokine families exhibit sequence similarity and show homology and some promiscuity in their reciprocal receptor systems. However, they have no functional similarity. Cytokine families also contain important regulatory cell membrane receptor-ligand pairs, indicating an evolutionary pressure that utilizes common structural motifs for different immune functions in higher mammals. The TNF/TNF receptor superfamily contains immunoregulatory cytokines, including TNF-α, lymphotoxins, and cellular ligands such as CD40L, which mediates B and T cell activation, and FasL (CD95), which promotes apoptosis. Similarly, the IL-1/IL-1 receptor superfamily contains cytokines, including IL-1β, IL-1α, IL-1 receptor antagonist, IL-18, and IL-33, which mediate physiological and host defense functions. However, this family also includes the Toll-like receptors, a series of mammalian pattern-recognition molecules that play a critical role in recognizing microbial species at the onset of innate responses.[4]

CYTOKINE RECEPTORS

Cytokines act on their target cells by binding to specific membrane receptors. Many cell functions are regulated by members of the cytokine receptor superfamily. Signaling by cytokine receptors depends upon their association with the *Janus* kinases (JAKs), which couple ligand binding to tyrosine phosphorylation of signaling proteins recruited to the cytokine receptor complex.[5] Among these signaling proteins is a unique family of transcription factors named the signal transducers and activators of transcription (STATs) (Figure 1). The receptors and their corresponding cytokines have been divided into several families based on their structure and activities, including:

Type I Cytokine Receptors

Type I cytokine receptors lack intrinsic protein tyrosine kinase activity and contain particular conserved patterns in their extracellular amino acid domain. This family includes the receptors for IL2 (beta subunit), IL3, IL4, IL5, IL6, IL7, IL9, IL11, IL12, GM-CSF, G-CSF, Epo, LIF, CNTF, and also the

TABLE 1: Due to the following properties, cytokines can regulate cellular activity in a coordinated, interactive manner.

Definition	Property
Pleiotrophy	One cytokine has many different functions.
Redundancy	Several different cytokines can mediate the same or similar functions.
Synergism	The combined effect of two cytokines on cell activity is greater than the additive effect of the individual cytokines.
Antagonism	The effects of one cytokine inhibit or compensate for the effects of another cytokine.

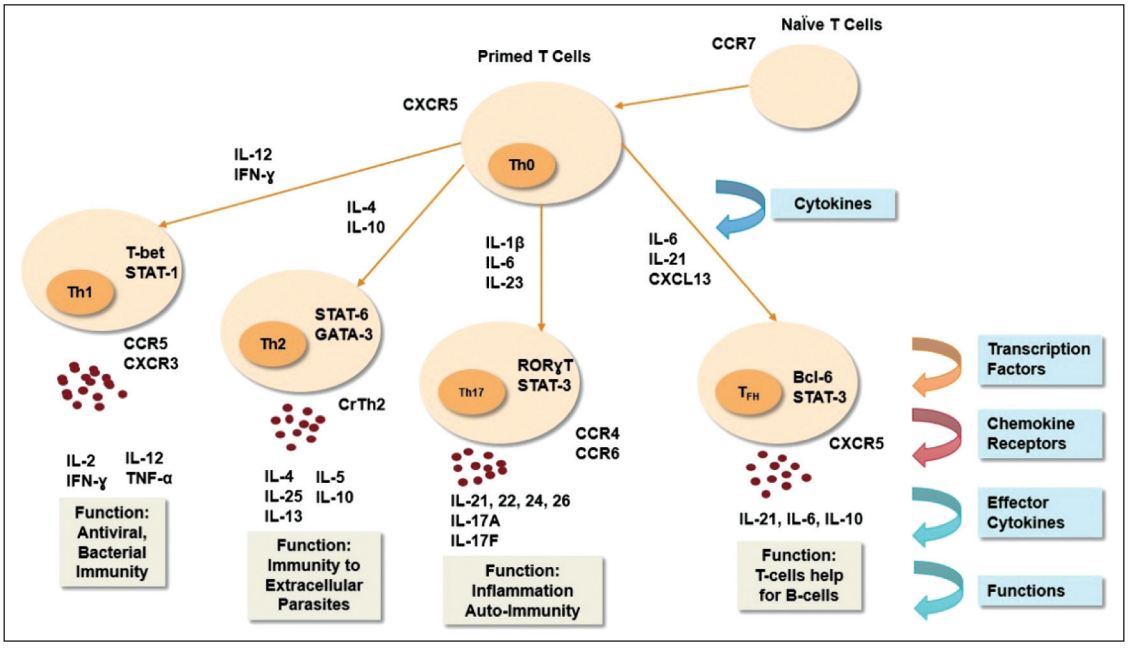


FIGURE 1: It often happens that different cell types secrete the same cytokine or that a single cytokine acts on several different cell types (pleiotropy). Cytokines are redundant in their activity, i.e., similar functions can be stimulated by different cytokines. They are often produced in a cascade, as one cytokine stimulates its target cells to produce further cytokines. Cytokines can also act synergistically or antagonistically.

receptors for thrombopoietin (TPO), prolactin, and growth hormone. The type I cytokine receptor family is divided into three subgroups based on the ability of the family members to form complexes with one of three different types of receptor signaling components (gp130, common beta, and common gamma-the gamma chain of the IL2 receptor).

Type II Cytokine Receptors

Type II cytokine receptors are multimeric receptors composed of heterologous subunits and are mainly receptors for interferons. This family includes receptors for IFN-alpha, IFN-beta, IFN-gamma, IL10, IL22, and tissue factors. The extracellular domains of type II cytokine receptors show structural similarities in their ligand-binding domain. Several conserved intracellular sequence motifs have been described, which probably serve as binding sites for the intracellular effector proteins JAK and STAT.

Chemokine Receptors

Chemokine receptors are G protein-coupled receptors with seven transmembrane structures that couple to G proteins for signal transduction. Chemokine receptors are divided into different families: CC chemokine receptors, CXC chemokine receptors, CX3C chemokine receptors, and XC chemokine receptors (XCR1).[6]

The Family of Tumor Necrosis Factor Receptors (TNFR)

The cysteine-rich domain (CRD), common to all members of the tumor necrosis factor receptor (TNFR) family, is an elongated protein consisting of three disulfide bonds surrounding a CXXCXXC core motif. TNFR is linked to procaspases via adaptor proteins (FADD, TRADD, etc.) that can cleave other dormant procaspases and initiate the caspase cascade that leads to apoptosis.

TGF-Beta Receptors

TGF-beta receptors are single-pass serine/threonine kinase receptors. TGF-beta receptors include TGFBR1, TGFBR2, and TGFBR3, which can be distinguished by their structural and functional properties.

FUNCTIONS IN THE IMMUNE SYSTEM

Cytokines that play an important role in the innate immune system include TNF-α, IL-1, IL-10, IL-12, IFN-α, IFN-β, IFN-γ, and chemokines. TNF-α produced by activated macrophages is a response to microbes, in particular to the lipopolysaccharide (LPS) of Gram-negative bacteria. It is an important mediator of acute inflammation. As a result, endothelial cells produce adhesion molecules and chemotactic cytokines that facilitate the recruitment of neutrophils and macrophages to the site of infection. TNF-α also acts

on the hypothalamus to induce fever, and it promotes the production of acute-phase proteins. Activated macrophages also release interleukin 1, another inflammatory cytokine. It promotes the activation, costimulation, and secretion of cytokines and other acute-phase proteins. Activated macrophages, B cells, and T helper cells all release interleukin 10. It is predominantly an inhibitory cytokine. Interleukin 10 attenuates immune responses by reducing antigen presentation, MHC class II expression, and the expression of co-stimulatory molecules on macrophages. Interleukin 12 is produced by activated macrophages, B cells, and dendritic cells. It induces the differentiation of Th cells into Th1 cells and enhances the cytolytic functions of cytotoxic T cells and NK cells. Interferon-alpha and interferon-beta are cytokines produced by macrophages, dendritic cells, and many other cell types. They promote resistance to viral pathogens and the increased expression of MHC class I. Interferon-gamma is an important cytokine that is mainly produced by Th1 cells and, to a lesser extent, by NK cells. It promotes the activation of APCs and

TABLE 2: Major cytokines of the immune system: cell sources, cell targets, and signaling pathways.

Cytokine	Signaling Pathways	Molecular Mass (kDa)	Primary Producing Cells	Primary Consuming Cells
IL-1β	NF-κB, JNK and p38 MAPK	17.5	Monocytes, epithelial cells	Macrophages
IL-2	JAK1, JAK2, STAT5	15.5	CD4+T cells, NK cells	T_{reg} Cells
IL-3	JAK2, STAT5	15	T cells, mast cells, eosinophils	HPCs, myeloid cells, lymphoid cells, erythroid cells
IL-4	JAK1, JAK3, STAT6	15	T cells, NKT cells, γδT cells, mast cells	B cells, T cells, macrophages
IL-5	JAK2, STAT5	13	T_H2 cells, mast cells, eosinophils, NK cells	B cells, eosinophils
IL-6	JAK1, STAT3, STAT1, STAT5	21	T cells, macrophages, fibroblasts, endothelial cells	B cells, T cells, thymocytes
IL-7	JAK1, STAT5	17	Bone marrow stromal cells, epithelial cells	T cells, B cells, DCs
IL-8	p38 MAPK-ERK-AKT, NF-κB and JAK-STAT (JAK2, STAT3)	8	Monocytes, T cells, neutrophils, fibroblasts, endothelial cells	Neutrophils
IL-9	JAK1, JAK3, STAT5, STAT3	16	Mast cells, T_H2 cells, T_H17 cells, T_H9 cells	T cells, B cells, mast cells, HPCs
IL-10	TYK2, JAK1, STAT3, STAT1	18.6	T_H2 cells, macrophages, DCs, B cells	T cells, macrophages
IL-11	JAK1, STAT3, STAT1	19	Bone marrow stromal cells	HPCs, stromal cells
IL-12	TYK2, JAK2, STAT4	34.7 (p40) and 22.5 (p35)	Activated macrophages, DCs	Activated T cells, NK cells
IL-13	JAK1, STAT6 and p38 MAPK	12.5	T_H2 cells	B cells, eosinophils, fibroblasts, mast cells, macrophages
IL-15	JAK1, JAK3, STAT3, STAT5	13	DCs, monocytes, epithelial cells	T cells, NK cells, NKT cells
IL-17	NF-κB, ERK1 and ERK2,	15.5	T_H17 cells, NK cells, NKT cells	Fibroblasts, endothelial cells, epithelial cells, keratinocytes, macrophages, DCs
IL-18	NF-κB, JNK and p38 MAPK	18	Macrophages	T cells, NK cells
IL-20	JAK1, JAK2, STAT3	17.6	Monocytes, granulocytes, keratinocytes, DCs, fibroblasts	Keratinocytes, endothelial cells, adipocytes
IL-21	JAK3, STAT3, STAT5	17	$CD4^+$T cells, T_h17 cells, NKT cells	B cells, NK cells, T cells
IL-23	TYK2, STAT3, STAT4, STAT5	55	DCs, macrophages, B cells, endothelial cells	T_H17 cells, γδT cells, NKT cells, ILCs
G-CSF	JAK1, JAK2, STAT3	18.8	Monocytes, macrophages	Neutrophils
GM-CSF	JAK2, STAT5	14	Activated T cells, NK cells, macrophages	Granulocytes, monocyte precursors
CSF1	PI3K-AKT, ERK1 and ERK2, and JAK-STAT	37	Fibroblasts, endothelial cells, stromal cells, macrophages, osteoblasts	Monocytes, macrophages
IFNα	TYK2, JAK1, STAT1, STAT2	19.2	pDCs, NK cells. T-cells, B cells, macrophages, fibroblasts, endothelial cells, osteoblasts	Macrophages, NK cells
IFNγ	JAK1, JAK2, STAT1	17	T cells, NK cells, NKT cells	T cells, monocytes, macrophages
TNF	NF-κB, JNK, ERK1 and ERK2, and p38 MAPK	17.5	T cells, B cells, NK cells, macrophages	T cells, B cells, endothelial cells
TGFβ	JNK, p38 MAPK and SMAU	25	T cells, macrophages	T cells

cell-mediated immunity and increases the expression of MHC class II molecules. IFN gamma has numerous functions in both the innate and adaptive immune systems. Chemokines are chemotactic cytokines produced by many types of leukocytes and other cell types. They belong to a large family of molecules that recruit leukocytes to sites of infection and play a role in lymphocyte trafficking.

Another group of cytokines that play a crucial role in the adaptive immune system are IL-2, IL-4, IL-5, TGF-β, IL-10, and IFN-γ.[7] Interleukin 2 is a type I cytokine that is mainly produced by T helper cells. It is the most important growth factor for T cells. It also promotes the growth of B cells and can activate NK cells and monocytes. Interleukin 4 is produced by T cells and mast cells. It stimulates the proliferation and differentiation of Th2 cells and, at the same time, inhibits the development of Th17. Interleukin 4 also stimulates the change of the Ig class to the IgE isotype. Interleukin 5 is a cytokine produced by Th2 cells. It promotes the growth and differentiation of B cells and eosinophils. Transforming growth factor Beta is a cytokine and a growth factor produced by T cells, macrophages, and many other cell types. It is primarily an inhibitory cytokine that inhibits the proliferation of T cells and blocks the action of pro-inflammatory cytokines. The cytokines presented are listed in Table 2.

INFLAMMATORY CYTOKINES

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines. Inflammatory cytokines can be divided into two groups: those that are involved in acute inflammation and those that are responsible for it. Inflammation, the tissue response to injury, is characterized in the acute phase by increased blood flow and vascular permeability, as well as the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines. Several cytokines play a key role in mediating acute inflammatory responses, namely IL-1, TNF-α, IL-6, IL-11, IL-8, and other chemokines such as G-CSF and GM-CSF.

The cytokines known to mediate chronic inflammatory processes can be divided into those involved in humoral inflammation, such as IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13, and transforming growth factor-β (TGF-β), and those that contribute to cellular inflammation, such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferons (IFNs), IFN-g inducing factor (IGIF), TGF-β, and TNF-α and -β (Figure 2). Inflammatory abnormalities are a large group of disorders that underlie a variety of human diseases. Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma.

Some cytokines exacerbate the disease (proinflammatory cytokines), while others serve to reduce inflammation and promote healing (anti-inflammatory cytokines) (Figure 3).

Proinflammatory Cytokines

A proinflammatory cytokine is a cytokine that promotes systemic inflammation. Interleukin (IL)-1 and tumor necrosis factor alpha (TNF-alpha) are proinflammatory cy-

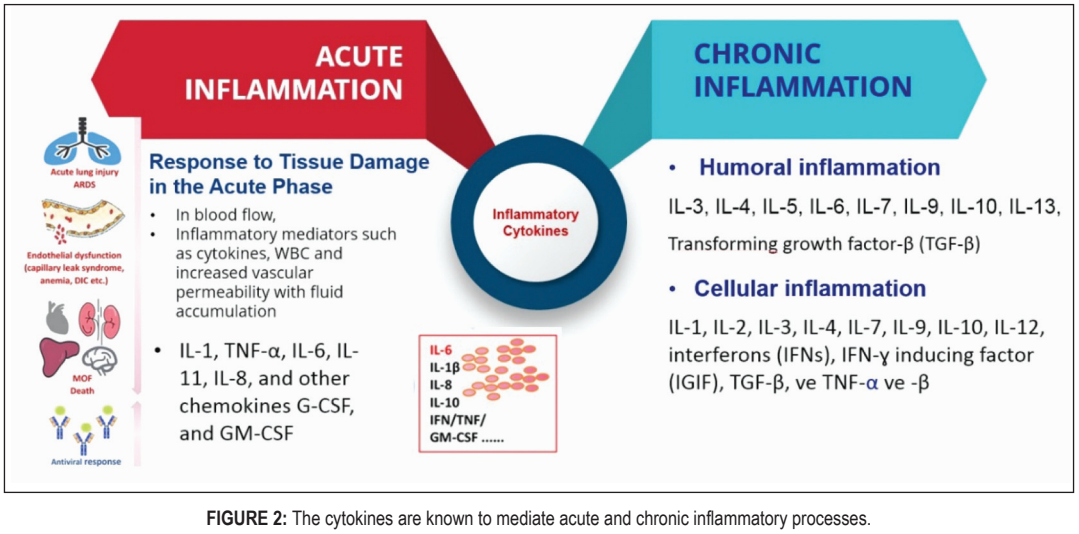


FIGURE 2: The cytokines are known to mediate acute and chronic inflammatory processes.

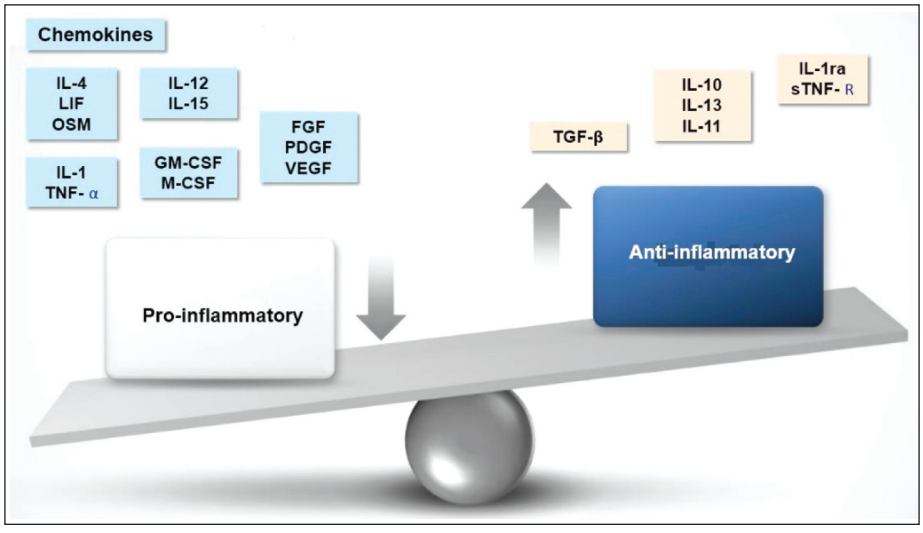


FIGURE 3: Proinflammatory and anti-inflammatory cytokines control the response to inflammation.

tokines.[8] When administered to humans, they lead to fever, inflammation, tissue destruction, and, in some cases, shock and death. Reducing the biological activities of IL-1 and TNF-alpha is achieved by different but highly specific strategies that include neutralizing antibodies, soluble receptors, receptor antagonists, and inhibitors of proteases that convert inactive precursors into active, mature molecules. Blocking IL-1 or TNF-alpha is very successful in patients with rheumatoid arthritis, inflammatory bowel disease, or graft-versus-host disease, but not in people with sepsis. Agents such as TNF-neutralizing antibodies, soluble TNF receptors, and IL-1 receptor antagonists have been infused into >10,000 patients in double-blind, placebo-controlled studies. Although the 28-day survival rate with anti-cytokine therapy was highly consistent (2 to 3%), the effect was not statistically significant.

Anti-İnflammatory Cytokines

The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the proinflammatory cytokine response. Cytokines interact with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Their physiological role in inflammation and their pathological role in systemic inflammatory conditions are increasingly recognized. Major anti-inflammatory cytokines include the interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13.[9] Specific cytokine receptors for IL-1, tumor necrosis factor-alpha, and IL-18 also function as proinflammatory cytokine inhibitors.

CYTOKINES AS DIAGNOSTIC MARKERS

INFLAMMATORY DISEASES

Cytokines have been extensively studied as diagnostic tools in certain inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease, cancer, and cardiovascular disease.[10-13] Infection, trauma, ischemia, and severe injury contribute to the pathogenesis of severe sepsis, which is characterized by an overwhelming production of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1b, and high-mobility group box (HMGB)-1. These cytokines trigger a positive inflammatory response that promotes local coagulation to limit tissue damage. However, the excessive production of these proinflammatory cytokines can be even more dangerous than the original stimulus by overcoming the normal regulation of the immune response and causing pathological inflammatory disorders. Sepsis also activates the production and release of specific anti-inflammatory substances, including cytokine receptor antagonists, soluble cytokine receptors, and anti-inflammatory cytokines. Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring inhibitor of IL-1 that competitively binds to the IL-1 receptor and inhibits the action of IL-1. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that likely has an important down-regulatory function in reducing the production of various proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. Theoretically, these anti-inflammatory substances could have an important regulatory function in controlling and attenuating the systemic inflammatory response in sepsis.[14-17]

BIOMARKER PANELS

The use of cytokine panels has shown promise in improving diagnostic accuracy and aiding in the differentiation of diseases with similar clinical features. Some of the most promising biomarker panels for diagnosing inflammatory diseases include a combination of cytokines and other inflammation-related markers. These panels can provide valuable insights into the underlying immune responses and inflammatory processes in various conditions.[18,19] Some of the commonly used and promising inflammatory markers are:

- Blood counts (e.g., white blood cell count, red blood cell count, hemoglobin count)
- C-reactive protein (CRP)
- Erythrocyte sedimentation rate (ESR)
- Procalcitonin
- Calprotectin

In addition to these markers, researchers are exploring other protein biomarkers for detecting various biological changes and using high-throughput techniques to identify new and/or better biomarkers for inflammatory diseases.[20] The development of biomarker-based clinical tests is a key challenge in personalized medicine, and the identification of ideal biomarkers holds the potential for improved diagnosis, prognosis, and treatment.[21] It is essential to note that the development and validation of these biomarker panels require further research and clinical trials to ensure their accuracy, specificity, and reliability in diagnosing and managing inflammatory diseases.[19]

PROGNOSTIC VALUE OF CYTOKINES

PREDICTING DISEASE OUTCOMES AND LONG-TERM IMPLICATIONS

Cytokines have been demonstrated to be promising prognostic indicators for disease progression and severity, as well as valuable biomarkers for understanding long-term implications and chronic inflammatory diseases. Some key findings from the search results include:

Cytokines can be used as biomarkers to indicate or monitor disease or its progress and may serve as clinically applicable parameters for therapies.[22]

A study found that interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels were independently predictive of patient outcomes in terms of both disease severity and survival in COVID-19.[23,24] In addition, high levels of pro- and anti-inflammatory cytokines, such as TNF, IL-10, IL-6, IL-8, and IL-2R, were correlated with the severity of COVID-19 and in-hospital mortality.[25] Therefore, these research findings indicate that cytokines are useful in estimating the severity of COVID-19 and aid in the distinction between patients who are critically ill and those who are less impacted.

Inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and inflammatory proteins and enzymes, can potentially serve as biomarkers for disease diagnosis, prognosis, and therapeutic decision-making.[26] Cytokines may improve patient outcomes when a definitive diagnosis is not possible, and the establishment of pathological cytokine profiles may aid in the differentiation of diseases with similar clinical features.[27]

Cytokines are representative of an individual's average level over time, and single measurements of selected cytokines are reliable for evaluating chronic inflammatory status in aging research.[28]

Further research is needed to establish the clinical cutoff levels and refine the predictive capabilities of cytokine panels in various conditions. Assessing cytokine profiles has shown potential in predicting the development and progression of chronic inflammatory diseases, offering opportunities for early intervention and personalized management.

CHALLENGES AND FUTURE DIRECTIONS

VARIABILITY IN CYTOKINE MEASUREMENTS

Cytokines are important mediators that monitor and regulate immune and inflammatory responses via complex networks and serve as biomarkers for many diseases. The quantification of cytokines is of great value in both clinical medicine and biology, as the values provide insights into physiological and pathological processes and can be used to support diagnosis and treatment. Analyzing the performance parameters of the biomarker to ensure that the test is repeatable, reliable, and has the right level of specificity and sensitivity is commonly referred to as the analytical validation phase of biomarker development. Figure 4 shows a schematic representation of the planning and management phases of cytokines as a biomarker development process.

Factors affecting the quantification of cytokines in biological fluids, native concentrations in various body fluids, sample processing, and storage, sensitivity to freezing and thawing, soluble cytokine receptors, and various ad-

vanced rapid cytokine detection technologies that are promising candidates for point-of-care or at-home usage were examined.[29] Recent advances in "*in vitro*" and "*in vivo*" assays, biosensors based on different signals, and intracellular to extracellular protein expression have also been reported. However, the quantification of cytokines as prognostic indicators of disease progression and severity poses several challenges, including variability in measurements, a lack of standardized cut-offs, and the need for improved detection methods.[30] Some of these challenges include:

Variability in cytokine measurements: Cytokine quantification can be affected by various factors, such as the choice of assay, antibodies, and experimental conditions, leading to inconsistencies in the results.[22,27]

Lack of standardized cutoff levels: Establishing clinical cutoff levels for cytokines is challenging due to the limited number of studies investigating their effectiveness, and the need for more research to narrow down diagnostic ranges.[22]

Complex relationships: Cytokines often have complex relationships with diseases, making it difficult to identify simple associations between specific cytokines and disease outcomes. Additionally, the constant discovery of new cytokines and their functions can complicate the selection of appropriate biomarkers.[27]

Inconsistent results: The roles of inflammatory cytokines in diseases are not always consistent, with some studies reporting associations between specific cytokines and disease outcomes while others do not.[27]

Limited clinical applicability: Although some cytokines have shown promise as prognostic indicators, many of them have not yet found their way from the bench to the bedside, limiting their clinical applicability.[27]

Despite these challenges, ongoing research and clinical trials aim to address these issues and improve the use of cytokines as prognostic indicators for disease progression and severity.

EMERGING TECHNOLOGIES

Emerging technologies for cytokine detection include the development of biosensors with optical detection modalities and nanomaterial-enabled signal enhancement. These biosensors exploit various nanomaterials and nanotechnologies to achieve real-time, sensitive, and specific detection of cytokines addressing challenges such as low cytokine concentrations, dynamics in cytokine secretion, and cytokine binding to other proteins in the sample. Additionally, advancements in bioassays, flow cytometry, Luminex bead-based assays, and electrochemiluminescent multiplex immunoassays have achieved success in detecting multiple cytokines in serum and plasma samples. Furthermore, methods for detecting and analyzing cytokines range from single-target ELISAs to large antibody arrays and microparticle multiplex assays.[13,23,30,31] Another approach involves the measurement of cytokine mRNA transcript abundance, allowing for the detection of many different cytokines from relatively small sample amounts.[32] However, these methods may have limitations, such as the possible underestimation of cytokine levels and the high cost associated with certain detection techniques.

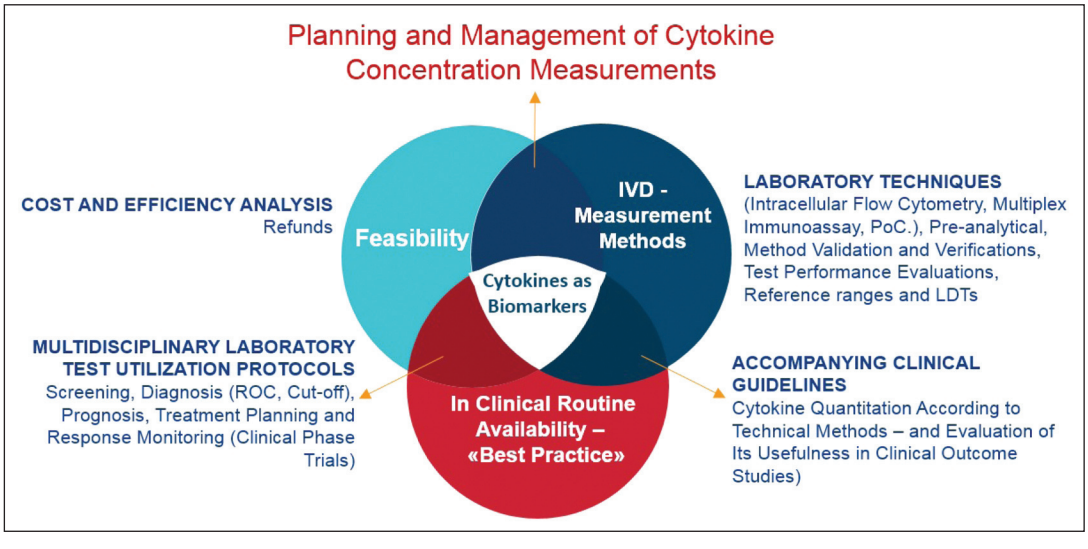


FIGURE 4: A biomarker is linked to clinical and biological endpoints through qualification, analytic validation, and clinical verification, a tiered evidence process.

PERSONALIZED MEDICINE

The consideration of individual variability in cytokine responses and the development of tailored diagnostic and therapeutic strategies represent an exciting avenue for future research and clinical practice.[33] Emerging technologies for improved cytokine detection include advancements in proteomic techniques, such as the development of antibodies and aptamers for cytokine molecule capturing.[34] These technologies aim to achieve more accurate and reproducible measurements of cytokines, addressing challenges such as low cytokine concentrations, dynamics in cytokine secretion, and cytokine binding to other proteins in the sample. However, the absolute cytokine concentrations should be interpreted with caution due to the potential for interference with other proteins/antibodies in the sample. Additionally, inter-assay comparison of different platforms for cytokine analysis has shown variability in quantitative results, emphasizing the need for careful testing and consideration of inter-individual and inter-assay variability. These advancements in proteomic techniques are expected to facilitate the identification of targets for personalized therapeutic approaches and contribute to the development of more effective treatments. However, further research and validation are needed to ensure the reliability and clinical applicability of these emerging technologies. Therefore, ongoing research and development aim to address these limitations and improve the reliability and clinical applicability of emerging technologies for cytokine detection.

CONCLUSION

In conclusion, cytokines have emerged as promising biomarkers for clinical utilization in monitoring and assessing inflammatory conditions, with potential applications in diagnostic, prognostic, and therapeutic settings. As diagnostic markers, cytokines can help differentiate specific inflammatory conditions, such as rheumatoid arthritis and inflammatory bowel disease, and improve diagnostic accuracy through biomarker panels. As prognostic indicators, cytokines can predict disease outcomes, progress, and severity, providing valuable insights into long-term implications and chronic inflammatory diseases. Furthermore, cytokine-targeted therapies have shown potential for treating inflammatory diseases and monitoring treatment response can serve as an indicator of therapeutic efficacy. However, several challenges remain in using cytokines as biomarkers, including variability in cytokine measurements, lack of standardized cutoff levels, complex relationships between cytokines and diseases, and limited clinical applicability. Despite these challenges, ongoing research and development aim to address these issues and improve the reliability and clinical applicability of cytokine biomarkers in clinical practice. Emerging technologies for cytokine detection, such as biosensors, bioassays, flow cytometry, and electrochemiluminescent multiplex immunoassays, offer new approaches for improved cytokine detection. These advancements have the potential to enhance the accuracy and efficiency of cytokine measurement, allowing for a better understanding of disease processes and personalized medicine considerations. However, further research and validation are needed to ensure the reliability and clinical applicability of these emerging technologies in the clinical utilization of cytokines as inflammation markers.

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Sepsis Biomarkers

Sepsis Biyobelirteçleri

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ABSTRACT Sepsis is a clinical syndrome that includes physiological, biological, and biochemical abnormalities due to the dysregulation of the host's response to infection. Sepsis and the resulting inflammatory response can lead to multiple organ dysfunction syndromes (MODS) and death. Early diagnosis and prompt antibiotic prescription are essential in treating bacterial sepsis. Providing a timely distinction between infectious and non-infectious patients is often challenging. Some biomarkers have been used to help diagnose sepsis and determine disease severity and prognosis. Unfortunately, a biomarker with sufficient sensitivity and specificity to diagnose or exclude sepsis has not yet been identified. About 200 different biomarkers related to sepsis have been studied. Several biomarkers have been identified for sepsis. Liquid Phase Pattern Recognition Molecules (PRMs), cytokines and chemokines, complement systems, Damage-Associated Molecular Pattern (DAMPs), cell membrane receptors, micro RNAs (miRNA), non-coding RNAs (ncRNA), cell proteins, metabolites, and soluble receptors are some of them. Classical biomarkers such as C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Procalcitonin (PCT) are the best known. However, the need to identify good combinations remains.

Keywords: Sepsis; biomarkers

ÖZET Sepsis, konakçının enfeksiyona verdiği yanıtın düzensizliğine bağlı olarak fizyolojik, biyolojik ve biyokimyasal anormallikleri içeren klinik bir sendromdur. Sepsis ve ortaya çıkan inflamatuar yanıt, çoklu organ disfonksiyon sendromlarına (MODS) ve ölüme yol açabilir. Bakteriyel sepsisin tedavisinde erken tanı ve hızlı antibiyotik tedavisi önemlidir. Enfeksiyöz ve enfeksiyöz olmayan hastalar arasında zamanında bir ayrım sağlamak genellikle zordur. Bazı biyobelirteçler, sepsis tanısına yardımcı olmak ve hastalığın ciddiyetini ve prognozunu belirlemek için kullanılmıştır. Ne yazık ki, sepsis tanısı veya dışlanması için yeterli duyarlılığa ve özgüllüğe sahip bir biyobelirteç henüz tanımlanmamıştır. Sepsis ile ilgili yaklaşık 200 farklı biyobelirteç incelenmiştir. Sepsis için çeşitli biyobelirteçler tanımlanmıştır. Sıvı faz modeli tanıma molekülleri (PRM'ler), sitokinler ve kemokinler, komplement sistemleri, Hasarla İlişkili Moleküler Modeller (DAMP'ler), hücre zarı reseptörleri, mikro RNA'lar (miRNA), kodlamayan RNA'lar (ncRNA), hücre proteinleri, metabolitler ve çözünür reseptörler bunlardan bazılarıdır. C-Reaktif Protein (CRP), Interleukin-6 (IL-6) ve Prokalsitonin (PCT) gibi klasik biyobelirteçler en iyi bilinenlerdir. Bununla birlikte, iyi kombinasyonları belirleme ihtiyacı devam etmektedir.

Anahtar Kelimeler: Sepsis; biyobelirteçler

SEPSIS

Sepsis has been defined as life-threatening organ dysfunction resulting from dysregulated host response to infection.[1] It is a heterogeneous condition in terms of physiological and immunological responses. A meta-analysis of 20 years of sepsis treatments showed a slow decline in mortality over time, although not statistically significant.[2] The problem is not limited to the first month, as about 40% of septic patients die within the first year.[3]

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Microbiological culture is the gold standard for diagnosis of infection, but blood culture results are usually available within 48-72 hours. Delays in diagnosis and uncertainty may hinder the initiation of standard treatments. It can also sometimes be a false negative.[4]

Sepsis is a significant problem that can be fatal, and a laboratory test or biomarker that will accurately diagnose or predict sepsis would benefit the patient. For example, a diagnostic biomarker can reduce inappropriate antibiotic use and be used for antibiotic management. The use of biomarkers in the direction of sepsis is still beginning.

BIOMARKERS IN SEPSIS

Biomarkers can be defined as indicators used to measure susceptibility and biological responses to disease, detect disease early, and characterize different types of illness. A biomarker can be used for more than one situation. Many sepsis biomarkers can be used to diagnose, guide antibiotics, and predict prognosis. Several biomarkers have been identified, such as Liquid Phase Pattern Recognition Molecules (PRMs), cytokines and chemokines, complement systems, Damage Associated Molecular Patterns (DAMPs), cell membrane receptors, micro RNAs (miRNA), non-coding RNAs (ncRNA), cell proteins, metabolites, and soluble receptors. C-reactive protein (CRP), Interleukin 6 (IL-6), and Procalcitonin (PCT) are the most studied.[5-7]

HUMORAL IMMUNE RESPONSE, CYTOKINES, AND CHEMOKINES

The humoral immune response consists of many components. Some of these components are Liquid Phase Pattern Recognition Molecules (PRMs). C-Reactive Protein (CRP), Serum Amyloid P (SAP), and Pentraxin 3 (PTX-3) are examples of PRMs.[8]

C-reactive protein (CRP) is synthesized by hepatocytes and stimulated by cytokines such as IL-6. With a half-life of approximately 19 hours, CRP rises slowly at the onset of sepsis.[9,10] CRP is one of the most widely used and researched biomarkers.[11] In addition, increases in CRP concentrations in septic patients, as a prognostic factor, indicate an increased risk of organ failure and death.[12]

Pentraxin (PTX-3) is secreted by dendritic cells, fibroblasts, macrophages, and mesangial and glial cells due to pathogenic or inflammatory stimuli.[8] It has been shown that PTX-3 levels are elevated at the onset of sepsis and increase with disease severity and the number of organ dysfunctions.[13] The combination of PTX-3, IL-6, Procalcitonin (PCT), and Lactate predicted 28-day mortality better than the Sequential Organ Failure Assessment (SOFA) in patients diagnosed with sepsis or septic shock.[14] Although lactate is widely used for early sepsis detection, elevated lactate levels are not considered specific for diagnosing sepsis.[15]

Complement activation markers (C4d, factor Bb, C3, C3a, and C5a) appear to be higher in sepsis patients than in healthy volunteers.[16]

Activation of Pattern Recognition Receptors (PRRs) causes the secretion of proinflammatory cytokines. These inflammatory mediators are required for adaptive immune response activation. IL-1β, IL-6, IL-8, MCP-1, IL-10, and plasminogen activator inhibitor 1 (PAI-1) levels are elevated in the acute phase of sepsis.[17] The most commonly used biomarker in studies is IL-6. IL-6 is a cytokine that plays pro-inflammatory and anti-inflammatory roles.[18,19] IL-6 levels are usually less than 5 pg/mL but may be higher than 500 pg/mL in patients with sepsis.[20] Serum IL-6 level rises within 1 hour and peaks rapidly within 2 hours after an infectious stimulus.[21] Especially in patients with Acute Kidney Injury (AKI), serum levels of IL-6 are more sensitive than PCT as a diagnostic marker.[22] High levels of IL-6 and IL-10 predict higher mortality for septic patients.[23]

DAMAGE ASSOCIATED MOLECULAR PATTERNS (DAMPs)

DAMPs are endogenous molecules released from stressed or damaged cells. It contributes to host defense but may also promote pathological inflammatory responses.

Calprotectin is a protein found in the cytosol of neutrophils and macrophages and is released under cell stress or injury. Calprotectin plasma levels are found to be high in sepsis.[24]

ENDOTHELIAL CELLS AND BLOOD-BRAIN BARRIER (BBB) MARKERS

These markers in protein or peptide structure can be evaluated as a marker of endothelial cells and BBB integrity.[25] Occludin (OCLN) and Zonula-Occludens 1 (ZO-1) values increase with disease severity in septic patients.[26] In studies, molecules were also evaluated such as soluble E-selectin (sE-selectin), soluble fms-like tyrosine kinase 1 (sFlt-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1) and PAI-1. All of these molecules have been associated with sepsis severity.[27]

Calcium-Binding Protein B (S100B) reflects BBB disruption and glial cell damage. It is found to be high in patients with sepsis-associated encephalopathy. It is positively correlated with IL-6 levels.[28]

In septic patients, Syndecan-1, Antithrombin, VCAM-1, PAI-1, E-selectin, HMGB-1, Histone-H3, IL-1β, IL-6, and IL-8 are increased compared to healthy controls. Syndecan-1 reaches higher levels in patients with a mortal course. Therefore, it is a reliable biomarker for predicting mortality.[29]

MEMBRANE RECEPTORS, CELL PROTEINS, AND METABOLITES

Cell surface receptors incorporated into the plasma membrane of cells act on cell signaling by binding to extracellular molecules. Differentiation cluster elements such as CD-13, CD14, CD25, CD64, and HLA-DR successfully predict mortality.[30] The combination of nCD64 and SOFA score is highly successful in predicting 28-day mortality. nCD64 expression is higher in septic patients and distinguishes a positive microbial culture well.[31]

CD14 is a glycoprotein found on the membrane surfaces of monocytes/macrophages. It acts as a receptor for lipopolysaccharide (LPS) and LPS-binding protein (LBP) complexes.[6] The sensitivity and specificity of PCT and Presepsin (sCD14 subtype) in the diagnosis of sepsis are close to each other.[32] TREM-1 levels are higher in children with septic shock.[33]

INTESTINAL PERMEABILITY MARKERS

In critically ill patients, an increase in intestinal permeability occurs. So SIRS and MODS can be triggered.[34] Plasma Zonulin and D-lactic acid levels are higher in sepsis patients.[35,36] Likewise, intestinal fatty acid binding protein (I-FABP) is higher in patients with sepsis and septic shock.[36]).

HORMONES AND PEPTIDE PRECURSORS

Procalcitonin (PCT) is synthesized by C cells in the thyroid. However, it is made by extrathyroidal neuroendocrine tissue during sepsis. In sepsis, the leading producers of PCT are macrophages and monocytic cells of different organs, especially the liver. It is considered an acute-phase protein like CRP. Unlike CRP, PCT elevations can also be seen in renal failure. Therefore, it is less specific in kidney disease as a biomarker.[6,37] Presepsin and PCT levels are higher in sepsis and septic shock than in non-septic patients.[38]

Adrenomedullin (ADM) is synthesized in many tissues, especially in the adrenal cortex. Mid-regional pro-adrenomedullin (MR-proADM) level measured within the first 24 hours after the diagnosis of sepsis was associated with 7- and 90-day mortality.[39] MR-proADM is potentially abolished by continuous renal replacement therapy (CRRT).[40]

N-terminal prohormone BNP (NT-proBNP) is an inactive prohormone produced by the heart under intracardiac pressure. NT-proBNP levels during the acute phase of sepsis may indicate the risk of impaired physical function and muscle strength in sepsis survivors.[41] NT-proBNP levels at the time of hospitalization are higher in those who are mortal than those who survive.[42]

PANCREATIC STONE PROTEIN

A new protein biomarker, pancreatic stone protein (PSP), is promising. Several lines of evidence suggest that PSP has a higher diagnostic performance than procalcitonin and C-reactive protein in identifying sepsis and a substantial prognostic value for predicting adverse outcomes in ICU admission.

Pancreatic stone protein levels rise before clinical signs, and symptoms of sepsis develop. Sensing distant organ damage and systemic stress, the pancreas responds by secreting PSP, especially in septic complications. PSP levels are below 20 ng/mL in uninfected adult patients and rise to several hundred ng/mL in patients with severe sepsis and even to several thousand ng/mL in case of septic shock.[43,44]

NON-CODING RNA AND microRNAs

Non-coding RNAs (ncRNAs) and micro RNAs (miRNAs) are being studied as sepsis biomarkers. For example, long noncoding metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1) and micro RNA (miR)-125a are increased in patients with sepsis. The lnc-MALAT1/miR-125a ratio shows an increased risk of 28-day mortality.[45] In sepsis patients, long non-coding RNA maternally expressed gene 3 (lnc-MEG3) increases, miR-21 expression decreases, and accordingly, the lnc-MEG3/miR-21 ratio increases. An increase in the lnc-MEG3 and ratio of lnc-MEG3/miR-21 predicts a high risk of sepsis, while an increase in miR-21 predicts a low risk of sepsis. Both miR-125a and miR-125b expressions are increased in sepsis patients. In addition, miR-125b is superior in predicting the risk of 28-day mortality in patients with sepsis.[46,47]

SOLUBLE RECEPTORS

The soluble trigger receptor expressed in myeloid cell-1 (sTREM-1) is a member of the TREM family. This receptor is potentially a promising biomarker as it can be meas-

ured in many different biological fluids.[48] Elevated soluble tumor necrosis factor receptor type 1 (sTNFR1) predicts 30-day mortality.[49]

Patients with sepsis have high levels of the soluble urokinase plasminogen activator receptor (suPAR). Some authors have noted that suPAR has higher specificity than PCT or CRP, which may help exclude sepsis in suspected cases.[50] Increased serum soluble programmed death ligand 1 (sPD-L1) levels were predictive of 28-day mortality.[51,52]

NEUTROPHIL-RELATED BIOMARKERS

High levels of Resistin in patients hospitalized in the intensive care unit indicate an increased likelihood of developing new organ failure. Likewise, high myeloperoxidase (MPO) levels indicate an increased risk of developing coagulation and kidney failure.[53]

NLCR can be used as a biomarker to distinguish confirmed bacterial infections from non-bacterial ones. Low NLCR values (<3.0) can be used to exclude bacteria as the etiological cause in critically ill patients.[15]

SEPSIS BIOMARKERS IN ANIMAL MODELS

Biomarkers have also been used to predict mortality from sepsis in animal models. The most common animal model of sepsis is cecal ligation and puncture in mice or rats.[54] Plasma IL-6 levels can categorize mice into high or low-mortality groups.[55] Antibiotics are more effective in mice with low IL-6 levels but do not reduce mortality in mice with very high IL-6 levels.[56]

CONCLUSION

Sepsis is a very complex clinical condition, and patients affected by sepsis have a heterogeneous phenotype. An ideal biomarker for sepsis should have high sensitivity and specificity and result in a short time. It should also be easily interpreted and require minimal interference if possible.

Current biomarkers for sepsis are, unfortunately, far from perfect at the moment. Sepsis biomarkers have been used primarily for diagnosis, to guide antibiotic therapy, and for prognosis. Among the most commonly used ones, PCT seems to be a more specific and better prognostic biomarker than CRP and IL-6. PCT is now the benchmark for sepsis diagnostic biomarkers, so it is essential to compare the performance of new sepsis diagnostic biomarkers with that of PCT.

Most studies to date have had small sample sizes. Different clinical settings, tests, and threshold values in studies make it difficult to compare results. The fact that some biomarkers are not widely used and costly contributes to the heterogeneity of studies. Combining biomarkers, rather than using them alone, improves their performance.

The pathophysiology of sepsis is very complex, highlighting the clinical picture's importance. While a single biomarker for highly complex disease is unlikely to solve everything, current studies are promising. Nevertheless, no technique is currently available for real-time bedside usage.

TABLE 1: Summary of the biomarkers most studied for sepsis diagnosis.[50]

	Number of	Number of	Study Population and Setting			
Biomarker	Studies	Meta-Analyses	Neonates	Adults, ED	Adults, ICU	Other
Procalcitonin	27	2	7	2	17	Adults, various wards = 2; Neonates and paediatrics, various wards = 1
Presepsin	19	4	11	2	8	Adults, various wards = 1; Paediatrics, various wards = 1
CD64	14	3	6	3	7	Adults, various wards = 1
CRP	12	0	6	1	5	0
IL-6	9	2	5	0	5	Non-clinical, experimental study = 1
sTREM-1	6	1	1	0	5	Neonates and pediatrics, various wards = 1
Lactate	4	0	0	2	1	Various patients, various healthcare settings = 1
NLCR	4	0	0	1	2	Febrile pediatrics, aged between 1 month and five years = 1
suPAR	3	1	0	0	3	Adults, ED or ICU

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The Role of Nuclear Medicine in Clinical Molecular Imaging of Inflammation

İnflamasyonun Klinik Moleküler Görüntülemesinde Nükleer Tıbbın Yeri

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ABSTRACT Current developments in biotechnology and widespread use of imaging modalities significantly improved diagnosis and management of inflammatory conditions. Nuclear Medicine (NM) and molecular imaging (MI), owing to their nature as physiologic based methods have a great potential to detect inflammation related alterations early in the beginning before the morphologic changes occur. Therefore, molecular NM modalities lead to positive findings at an earlier period than the radiologic methods based on the morphologic tissue changes. Two main modalities in NM, SPECT (single photon emission tomography) and PET (positron emission tomography) can be used for *in vivo* identification of inflammation with different radiopharmaceuticals. Molecular imaging is generated through a variety of radiopharmaceutical agents targeting different cellular subtypes in the dynamic course of inflammation. Radiolabeled leucocytes, 18F FDG reflecting increased glucose metabolism, radiolabeled chemokine receptors and fibroblast activating proteins already have been molecular tracers in clinical practice. Currently hybrid imaging such as SPECT/CT and PET/CT providing anatomic and functional information together improved diagnostic accuracy and enhanced the value of NM and molecular imaging in inflammation.

Keywords: Inflammation; diagnostic imaging; radionuclide imaging; scintigraphy

ÖZET Günümüzde giderek yaygınlaşan moleküler görüntüleme yöntemleri yangısal süreçlerde tanı, izlem ve tedavi seçeneklerini belirlemede etkin olarak kullanılmaktadır. Nükleer Tıp (NT) yöntemleri tüm patolojik süreçlerde olduğu gibi erken dönemde, henüz yapısal bozukluklar oluşmadan önce, fizyolojik ve metabolik değişiklikleri göstererek moleküler görüntülemede (MG) önemli potansiyel oluşturmaktadır. Bu nedenle NT ve MG etkinliği morfolojik değişikliklere dayanan radyolojik görüntülemelere göre erken dönemde pozitif sonuç vermektedir. SPECT (tek fotonlu bilgisayarlı komputerize tomografi) ve PET (pozitron emisyon tomografi) olarak iki alt grupta incelenen NT görüntüleme yöntemlerinde farklı radyofarmasötikler kullanılarak yangısal sürecin *in vivo* olarak değerlendirilmesi mümkündür. İnflamasyonda rol oynayan farklı hücre alt gruplarının varlığı, sürecin dinamik yapısı, zamansal etkiler nedeniyle görüntülemede çok çeşitli moleküler hedeflerle görüntüleme mümkündür. Radyoaktif işaretli lökosit sintigrafisi, inflamasyonda artmış glukoz metabolizmasını hedefleyen F-18 FDG, radyoaktif işaretli kemokin reseptörler ve fibroblast aktive edici proteinler klinik kullanımda yer almaktadır. NT görüntüleme sistemlerinin BT ile hibritleşmesi anatomik ve fonksiyonel-metabolik bilgileri bir araya getirerek MG de yeni bir kapı açmış ve tanısal katkı önemli ölçüde artmıştır.

Anahtar Kelimeler: İnflamasyon; tanısal görüntüleme; radyonüklid görüntüleme; sintigrafi

The identification and localization of an infective or inflammatory process, particularly early in the beginning, constitute an important role for clinical therapeutic management. In addition to clinical evaluation of the patient, various laboratory tests and imaging techniques including ultrasonography, CT, MRI, endoscopic techniques have been widely used. Nuclear medicine methods owing to their nature as physiologic based modalities have great potential to detect inflammation related alterations early in the beginning before the morphologic changes occur. Therefore, molecular NM modalities include PET, planar scintigraphy and SPECT imaging

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lead to positive findings at an earlier period than the radiologic methods based on the morphologic tissue changes. However, all these techniques have specific advantages and disadvantages related to the availability, cost and diagnostic accuracy. Magnetic resonance imaging (MRI), computerized tomography (CT) and conventional X-ray methods, based on the detection morphologic changes have good resolution properties but lack in diagnostic efficiency in the early period of inflammation before the structural alterations are occurring. Ultrasonography which is a less expensive and widely available method without radiation exposure has the disadvantages of being an operator-dependent modality and limitations in structures deep within the body.

Although radiologic methods described previously provide good spatial resolution, nuclear medicine and molecular imaging provide assessment of disease activity based on physiologic and metabolic alterations caused by underlying pathology. Those radiopharmaceuticals used in infection/inflammation imaging should be designed to provide good imaging quality, easy preparation, a reasonable cost and high diagnostic sensitivity.[1-4] While a variety of tracers have been developed, not all of them have been approved for clinical use in human. Those most common agents can be divided in different subgroups according to the molecular targets activated during the pathophysiological processes or according to the imaging modalities such as SPECT or PET. In this review, those nuclear medicine methods and molecular tracers will be discussed in 2 subgroups as conventional scintigraphy/SPECT and PET imaging tracers (Figure 1).

CONVENTIONAL NUCLEAR MEDICINE AND SPECT IMAGING

The recent developments in imaging systems improved the spatial resolution of conventional planary scintigraphy and higher diagnostic accuracy was provided with SPECT systems. Moreover, by combining SPECT systems with a CT component, hybrid systems provided correlation of anatomic images with functional and metabolic information on a single session of scanning. Currently, the most commonly used radiopharmaceuticals used in scintigraphic imaging of infectious and inflammatory diseases are radiolabeled white blood cells, radiolabeled murine monoclonal anti-granulocyte antibodies (MAB) and antibody fragments and infection specific agents such as labeled antimicrobial peptides and antimicrobial agents. Several adhesion molecules, integrins and selectins have also been radioactively labeled for infection-inflammation imaging studies.

^{99m}Tc labeled nanocolloids and human immunoglobulins (HIG) labeled with ^{99m}Tc or ^{111}In has also been used to image infection or inflammation. Increased capillary permeability, binding Fc part of IgG to Fc receptors on granulocytes, and binding of IgG to bacteria are postulated as responsible mechanisms. Radiolabeled HIG has been shown to be a good option for the evaluation of fever of unknown origin in particular immunodeficient patients. Tc-HIG imaging is also useful in inflamed joints in a greater extent than other anatomical imaging methods.

In the past, ^{67}Ga citrate has also been used in infection imaging but now it has been replaced with novel radioactive tracers of due to its unfavorable imaging properties. Therefore, ^{67}Ga imaging is not included within the scope of this review.

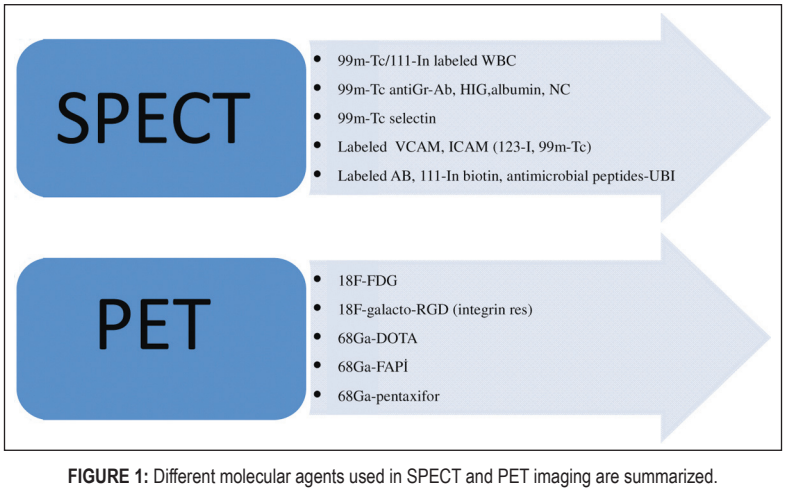


FIGURE 1: Different molecular agents used in SPECT and PET imaging are summarized.

RADIOLABELED WHITE BLOOD CELLS

In the mid-1970s, McAfee and Thakur investigated several methods for *in vitro* labeling of white blood cells.[5-7] They finally documented that Indium-111 and Technetium-99m complexes of oxine were the most appropriate molecular agents for cell labeling. Thereafter, autologous white blood cells labeling has gained wide acceptance in clinical nuclear medicine applications and guidelines published to improve interpretation of this method.[8]

The process of *in vitro* labeling of leucocytes with both radionuclides is a labor intensive process that should be completed under sterile conditions. The procedure is performed by obtaining a certain volume of the patient blood and then labeling leucocytes following several steps and isolating from the other cellular components of blood. Finally, labeled leucocytes are reinfused into the patient.[9] The uptake of labeled white blood cells is related to the specific migration to the site of infection and inflammation. Since the majority of labeled cells are neutrophils, this method is mostly indicated in bacterial infections. While ^{111}In and ^{99m}Tc as radionuclide options have their own advantages or disadvantages, in general, radiolabeled white blood cells have been successfully used in orthopedic infections, in diabetic foot, soft tissue infections including prosthetic vascular grafts and abdominal infections.[3,9-16] Particularly, labeled leucocytes imaging in combination with bone and bone marrow scintigraphy has been well validated in differentiation of infected prosthesis from aseptic loosening. It is useful to direct clinical management in which different therapeutic approach is required.[8,12] Moreover, favorable results were reported also in infectious disorders of cardiac implantable electronic devices, prosthetic valves and infective endocarditis.[17] Also, during the last years, with the advent of SPECT/CT systems, hybrid infection imaging with labeled cells provided higher diagnostic accuracy because of better resolution and morphological information.[18]

RADIOLABELED MONOCLONAL ANTI-GRANULOCYTE ANTIBODIES (MAB) AND ANTIBODY FRAGMENTS

As *in vitro* labeling of white blood cells require significant effort and might be impractical for certain clinical conditions, different *in vivo* labeling methods have been developed including anti-granulocyte antibodies and antibody fragments. The labeling of monoclonal antibodies with ^{99m}Tc can be considered as simple and easily applicable to routine imaging for *in vivo* labeling of granulocytes. It can be recommended in severely leucopenic individuals and also in young children where *in vitro* white blood cell labeling might be difficult.

The most common products in clinical use are a whole murine IgG anti-NCA-95 antibody (Besilesomab, Scintimun®), and an antibody fragment of class IgG1 class binding to normal cross-reactive antigen 90 present on leucocytes Fab'fragment anti-NCA-90 (Sulesomab, Leukoscan®).[19,20] However, whole murine antibodies may induce HAMA response (human anti-mouse antibodies) and therefore their use can be considered as impractical due to changed biodistribution when patients are reinvestigated. Sulesomab, being a Fab'fragment, can also be used and has the advantage of not inducing HAMA production. On the other hand, this agent is licensed in Europe only for clinical use in peripheral musculoskeletal infections. Also in similar to radiolabeled white blood cell imaging, some problems may appear in chronic low grade infections with radiolabeled anti-granulocyte antibody and fragments imaging. Moreover, false positive results may be noted in postoperative reactive granulation tissues.

INFECTION SPECIFIC MOLECULAR IMAGING AGENTS

In order to detect infection specifically rather than to detect host response, some investigators studied on antibiotics and antimicrobial peptides incorporating into the microorganisms therefore, accurately and specifically localizing infection. Antimicrobial peptides (AMP), produced by phagocytes, epithelial cells, endothelial cells, and many other cell types are small molecules with cationic charge. They serve as important component of natural immunity against infection by bacteria, viruses, fungi other pathogens during inflammation or microbial challenge. The most well-known AMP has been radiolabeled synthetic fragments of ubiquicidin (UBI), a murine antimicrobial peptide present in the cytosolic fraction of macrophages. Technetium-99m labeled UBI has been found to be both sensitive and specific for some infections, and also has shown promise for monitoring response to treatment.[21,22]

^{99m}Tc labeled ciprofloxacin (Infecton) has been one of the initial antimicrobic agent used in infection imaging.[23] Ciprofloxacin, as with other fluoroquinolones is a broad spectrum antibiotic agent which acts by trapping the type II topoisomerases, DNA gyrase and topoisomerase IV on DNA. Experimental studies showed that infection localizes in high concentrations in abscesses caused by both gram-positive and gram-negative bacteria. However, it does not accumulate in areas of sterile inflammation or abscesses with dead bacteria. So it has been documented that infection imaging provides clinical useful results over labeled white blood cell imaging.[24]

PET AGENTS FOR MOLECULAR IMAGING OF INFECTION AND INFLAMMATION

PET imaging has been a common modality in molecular and metabolic imaging of many malignant and benign clinical conditions. Flourodeoxyglucose, FDG, the most traditional agent in positron emission imaging for oncologic diseases, has also wide gained wide acceptance in the evaluation of inflammatory diseases. FDG is a glucose analogue that is primarily taken up by high-glucose-consuming highly proliferating malignant cells and inflammatory cells. It is rapidly transported via glucose transporters (GLUTs) into the cytosol, phosphorylated by hexokinase, and intracellularly trapped as FDG-6-phosphate. Phosphorylation by hexokinase prevents metabolization of FDG until radioactive decay. After radioactive decay, it is converted to glucose-6-phosphate and metabolized in the same way as normal glucose. It is shown that FDG accumulation depends on the rate of transport into the cytosol, the enzymatic activity of hexokinase, and the rate of dephosphorylation in the tissue. It is also noted that phagocytic cells such as neutrophils and monocytes/macrophages, are also able to express high levels of glucose transporters, especially GLUT-1 and GLUT-3, as well as hexokinase activity.[25] Therefore, the use of FDG PET imaging has been validated in several inflammatory conditions fever of unknown origin, granulomatous diseases such as sarcoidosis or tuberculosis (Figure 2).[26,27]

Particularly, ^{18}F FDG PET imaging has a key role in clinical management of adults and children with a fever of unknown origin, in whom clinical and laboratory findings together with conventional imaging methods fail to identify diagnosis. The published data also support its clinical value in cardiovascular infections, including infective endocarditis, as well as cardiac implantable electronic device and prosthetic vascular graft infections.[26-29]

^{18}F FDG has also been used in *in vitro* labeling of human leucocytes. Copper-64 and and zirconium-89 have also been studied in white blood cell labeling for PET imaging of infection. However, these agents currently do not appear to be validated in clinical practice.

PROMISING MOLECULAR AGENTS BEYOND FDG

In addition to ^{18}F FDG there have been new potential inflammation imaging targets in PET imaging. Some of these biomolecular agents are currently under investigation while some have already have showed clinical promising results.[30,31] The most representative targets of molecular imaging in inflammatory diseases can be are summarized as

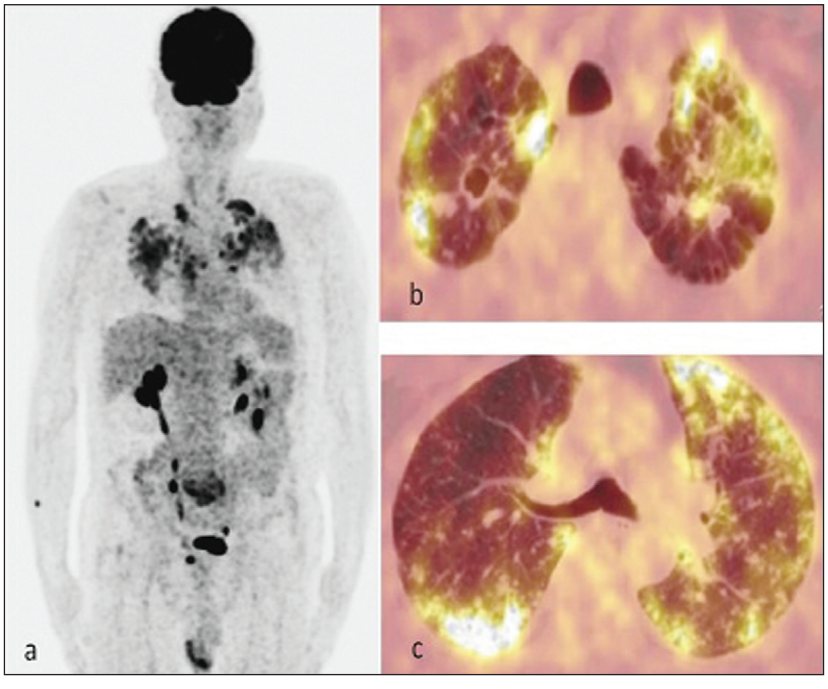


FIGURE 2: A 68 years old man undergoes oncologic FDG PET/CT imaging for the evaluation of colorectal tumor. Left column **(a)** Maximum intensity projection (MIP) image shows increased FDG accumulation in both lungs. Transaxial fused lung parenchyma images **(b, c)** through the upper lobes indicate cavitary lesions and multiple tiny nodular infiltrations with hypermetabolic activity due to pulmonary tuberculosis.

chemokine receptors, somatostatin receptors and fibroblast related peptides.

Chemokine receptors: Chemokine receptors of different subtypes are biologically active peptides expressed in pro-inflammatory cells. Their primary function is to induce cell migration. So they have potential as biologic target for infection imaging. With this aim, ^{68}Ga labeled -Pentaxifor, targeting CXCR group chemokine receptors has already been used in various infectious and inflammatory diseases. While it has originally been developed for tumor imaging, particularly for myeloproliferative diseases and some glial tumors, pentaxifor imaging showed successful results in chronic bone infections also in imaging of post-myocardial tissue inflammation.[32,33]

Somatostatin receptors: Somatostatin receptors (SSTR) have been investigated as a target for neuroendocrine tumors. As high level of SSTR expression was also documented on activated lymphocytes and macrophages, this receptor has the potential to be used as a new target for inflammation imaging. ^{68}Ga labeled PET tracers (DOTATOC, DOTANOC, and DOTATATE) to target SSTR in inflammatory disorders were utilized in some inflammatory diseases mainly in postinfarction cardiac inflammation and sarcoidosis.[34,35]

Fibroblast activated proteins: Fibroblast activation protein (FAP) is a type II membrane-bound glycoprotein which is overexpressed in tumor associated fibroblasts and activated fibroblasts found at wound healing/inflammatory sites. FAP directed PET imaging has been introduced for the evaluation of FAP expression in stromal microenvironment of a tumor. The current literature also indicate the role of FAPI PET imaging in a variety of inflammatory conditions including IgG related disease, post MI inflammation, rheumatoid arthritis and sarcoidosis.[36,37] It has also a potential role for risk stratifying of patients with inflammatory or fibrotic diseases.

CONCLUSION

Nuclear medicine and molecular imaging with a variety of different biologic markers has an important role in the evaluation of infectious and inflammatory diseases. These modalities provide early identification of the biologic processes and serve as noninvasive diagnostic methods while providing prognostic value and contributing to the therapeutic decision making. The new developments in imaging systems, combination of CT component into the SPECT or PET systems enhance the diagnostic accuracy. Finally, research studies focusing on developing the most ideal molecular agents for infection and inflammation imaging hold great promise for the future of nuclear medicine.

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Clinical Molecular Imaging in Inflammation with Radiological Perspective

Radyolojik Bakışla Yangıda Klinik Moleküler Görüntüleme

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ABSTRACT Radiology has imaging methods that are specific and non-specific to inflammation. The cellular and fluid changes revealed by the inflammatory process produce direct or indirect findings to imaging methods, and these findings are used in the differential diagnosis. Especially in magnetic resonance imaging, perfusion changes, imaging of the inflamed vessel wall and changes in inflammatory bowel disease, and inflammation imaging with hyperpolarized contrast agents are frequently used methods with the sequences developed in recent years. It also contributes to the sensitive diagnosis of inflammation with diffusion-weighted imaging. In this presentation study, information about the details of the subject will be shared with examples.

Keywords: Inflammation; imaging; radiology

ÖZET Radyoloji yangıya özgü olan ve olmayan görüntüleme yöntemlerine sahiptir. Yangı sürecinin ortaya çıkardığı hücresel ve sıvısal değişiklikler görüntüleme yöntemlerine doğrudan ve ya dolaylı bulgular üretir ve ayırıcı tanıda bu bulgulardan yararlanılır. Özellikle manyetik rezonans görüntülemede son yıllarda geliştirilen sekanslarla perfüzyon değişiklikleri, yangılı damar duvarının görüntülenmesi, yangısal barsak hastalığındaki değişikliklerin ortaya konması ve hiperpolarize kontrast maddeler ile yangı görüntülemesi sıklıkla kullanılan yöntemlerdir. Difüzyon ağırlıklı görüntüleme de yangının duyarlı tanısına katkı sağlar. Bu sunumda örneklerle konunun ayrıntıları hakkında bilgiler paylaşılacaktır.

Anahtar Kelimeler: Yangı; görüntüleme; radyoloji

The repair process of damage to tissues for any reason is called inflammation. This damage usually is a result of a trauma, in some cases microorganisms are responsible from this process. The pattern of damage is recognized by the receptors and chemical agents such as histamine, complement, kinin and prostaglandin are released into the environment. As a consequence of this, dilatation of the vessels and increased permeability of the capillaries occur, while neutrophils migrate to the region and the number of white blood cells increases. The purpose of vasodilation is to create hyperemia in the area. This is also the cause of the increase in temperature and redness in the inflamed area. In this way, oxygen and other nutrients come to the region and increase the metabolic activity.[1]

As a result of increased capillary permeability, exudate is formed in the environment. Protein and coagulation factors also leak and blood flow rate decreases. Edema occurs with protein leakage. Swelling and pain appears as the main symptoms of inflammation . This means restriction of movement. The activation of coagulation factors provides fibrin formation. Neutrophils and leukocytes, on the other hand, contribute to healing by cleaning the damaged cells.[2]

These events, which develop at the molecular level, have various reflections in radiological imaging and these findings are used in the diagnosis of inflammation. These

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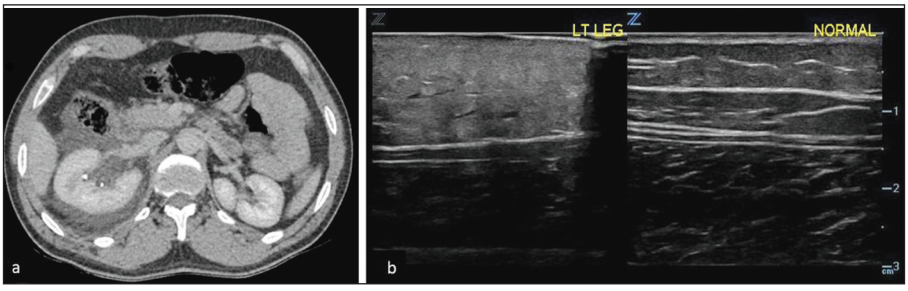


FIGURE 1: a: On computed tomography, inflammation around the right kidney shows an increase in density due to intense cell migration. **b:** Ultrasonography performed at leg level shows increased echogenicity due to inflammation on the left.

findings differ according to the imaging tool. For example, intense cell migration to the damaged area appears as an increase in echogenicity in ultrasonography and an increase in density in computerized tomography (CT) (Figure 1a, b).

Changes at the molecular level can be visualized more sensitively on magnetic resonance imaging (MRI). For example, since the movement of water molecules is restricted in diffusion-weighted imaging in an environment with increased cells, it is reflected in the image as an increase in intensity. Diffusion MR imaging is based on the mathematical representation of the random motion of water molecules between cells, called Brownian motion. Under normal conditions, water molecules continue to circulate with a certain speed and deflection in an unrestricted environment. As seen in inflammation, cell aggregation in the damaged area and the presence of infectious agents restrict this circulation. Restricted motion is shown as increased signal intensity. A map is obtained from the data obtained at different magnetic field intensities and this is called the ADC map. Its unit is mm^2/s. The values obtained from ADC maps also give information about the type of tissue in the measured area.[3]

Edema, which is a component of inflammation, also occurs with findings such as increased volume and effacement of tissue boundaries. In MRI, the density of water molecules in the edema area appears as an increase in intensity in T2-weighted series (Figure 2).

The increase in vascularity in the inflammation creates findings that contribute to the diagnosis by affecting the collection and transmission of the contrast agent in the contrast-enhanced studies (Figure 3 a,b). There are also special MRI scans developed for vessel wall imaging. It is possible to better evaluate the vessel wall by suppressing the blood in the flow. This technique is called Black Blood MR Imaging.[4] The most commonly used technique for this purpose: pre-and post contrast 3D T1-weighted fast spin-echo sequence (3D T1 FSE) (Figure 4).

It is also possible to visualize the impaired perfusion with radiological tools. In this regard, especially the sequences developed in MRI are used (Figure 5 a-d). Perfusion is the transport of oxygen and nutrients from the blood to the tissues via capillaries. It is possible to map perfusion and show hemodynamics in MRI. Blood flow and volume can also be calculated with MRI. The rate of progression of a blood cell can be determined by its mean transit time. Perfusion can be evaluated by two methods in MR.[5,6] The first of these is external influence. The model used in this method aims to determine the intravascular restriction and can be done in two ways. In dynamic imaging, the flow of contrast material given as a bolus over time is measured with very fast imaging (echo planar). In steady-state imaging, evaluation is made with images taken when the concentration in the blood is stabilized after a fixed infusion. In perfusion study with intrinsic effect (arterial spin labeling), the model aims to determine intravascular molecular motion. The motion of magnetically marked water protons is evaluated.

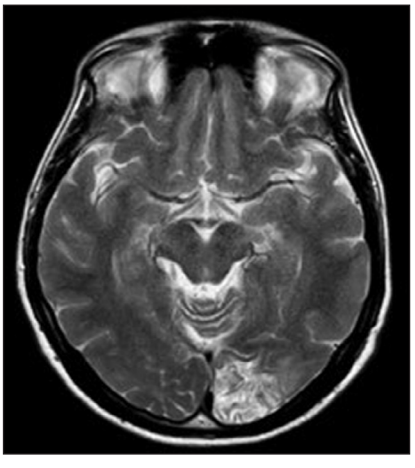


FIGURE 2: In the T2-weighted MR section, an increase in intensity in the edema area is observed in the left occipital lobe.

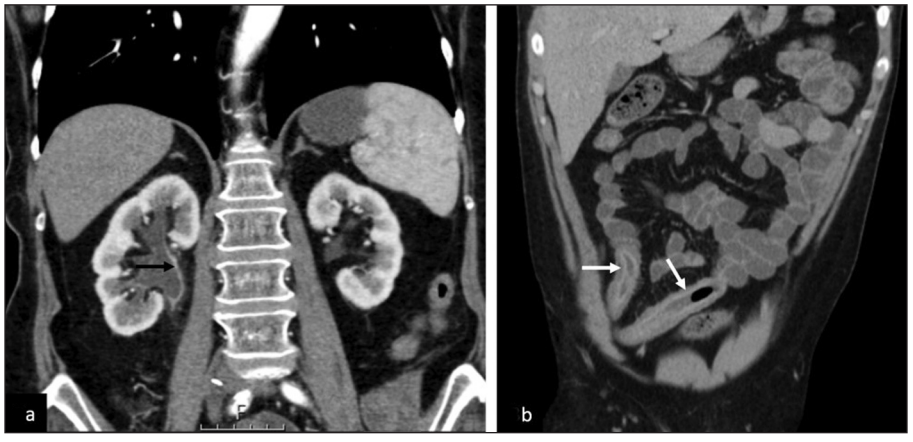


FIGURE 3: a: In the coronal plane CT section, contrast enhancement is seen in the epithelium as a result of infection in the right kidney collecting system (pyelitis). **b:** Disease activity at the terminal ileum level in a patient with Crohn's disease caused intense mucosal enhancement.

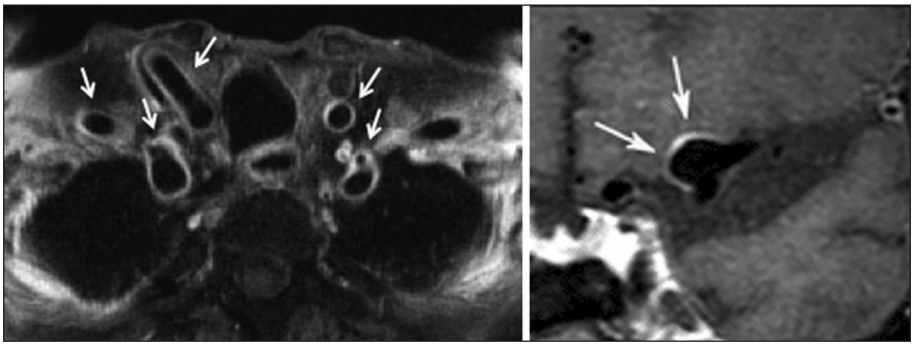


FIGURE 4: In the images obtained by black blood MR imaging technique, the vessel wall can be observed in detail.

Lymphatic movement or stasis enlarged lymph nodes with the arrival of inflammatory materials to the lymph nodes can also be seen in the images. As a result of lymphatic stasis, the lymphatic pathways enlarge and appears as streaking. In cases of severe inflammation, when an abscess occurs, it is easily observed with imaging modalities. A typical abscess has a thick wall that enhances after contrast administration (Figure 6).

In addition to the primary findings of the inflammation, the patterns formed in the tissues and clinical information also play an important role in the diagnosis. Diagnostic options can be reduced more easily in inflammations with a special pattern. One of these patterns is the coronavirus disease-2019 (COVID-19) pneumonia, which we have been familiar with in recent years (Figure 7). It presents with triangular patchy peripheral involvement in

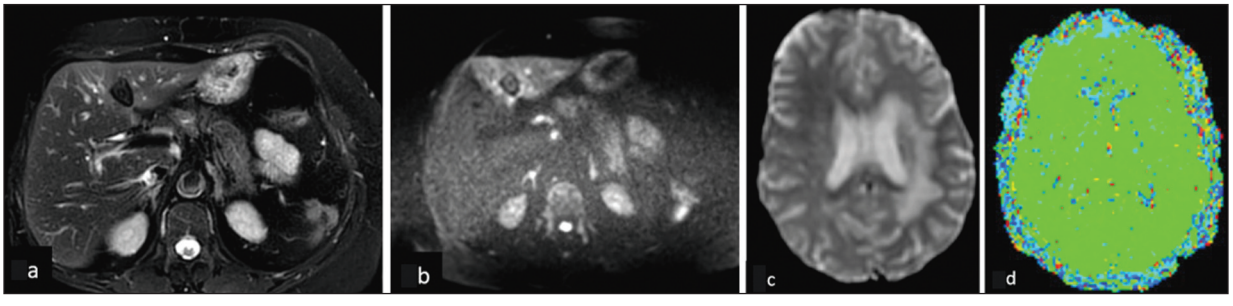


FIGURE 5: a: Perfusion disorder in the irrigation area due to thrombus in the left hepatic branch of the portal vein is not evident in the T2-weighted series. **b:** In the diffusion-weighted series, signs of restriction in the impaired perfusion area are observed. **c:** In the brain MRI section, there is a hypertense lesion area in the left parietooccipital area. **d:** Perfusion evaluation shows impaired perfusion in the area.



FIGURE 6: CT imaging of the abscess formed in the left due to pancreatitis. The abscess wall, its contents and the air it contains can be observed.

a ground glass pattern in both lungs.[7] The cavitary nodules observed in patients with tuberculosis, the fungus ball observed in the cavity in aspergillosis, the membrane structures and cyst wall qualifications in hydatid cyst infection, the stratification due to submucosal edema in acute cholecystitis and appendicitis, the presence of fat necrosis in the lesion center in epiploic appendicitis are some other pathologies with a special pattern.

There are also studies aiming to benefit from MR Spectroscopy, another diagnostic tool, in inflammatory imaging. MR spectroscopy is a method developed to detect biochemical components in tissue *in vivo*. The distribution of electrons in the atom causes the nucleus of each molecule to create a different magnetic field, thus imaging is provided by utilizing the individual resonance frequency of each molecule.[8] Spectroscopy studies can be performed for protons, carbon-13 and phosphorus-31. During imaging, water is suppressed, and a better image can be obtained. The most common change in pathological conditions is the decrease in NAA (N-acetylaspartate) and the increase in choline (Figure 8). In infestations such as toxoplasmosis, choline is reduced or absent.[9] It facilitates the differential diagnosis with lymphoma where these values are elevated.[10] Its main disadvantage is its low chemical and spatial sensitivity and long examination times.

One of the methods developed for inflammation imaging is the hyperpolarized MR technique.[11] In nature, protons are of low polarization. Protons polarized in different directions in the magnetic field cancel each other's magnetic effect. The remaining difference is the source of the MR signal. To reproduce this difference, hyperpolarized contrast agents were produced. Gases as well as hyperpolarized metabolites have been produced and used.[12] The first applications were made in the early 2000s. Metabolite measurements are done after the hypopolarized substance given in the examination. In the examinations performed with hyperpolarized 13C, the increase in pyruvate may indicate the severity of inflammation in arthritis

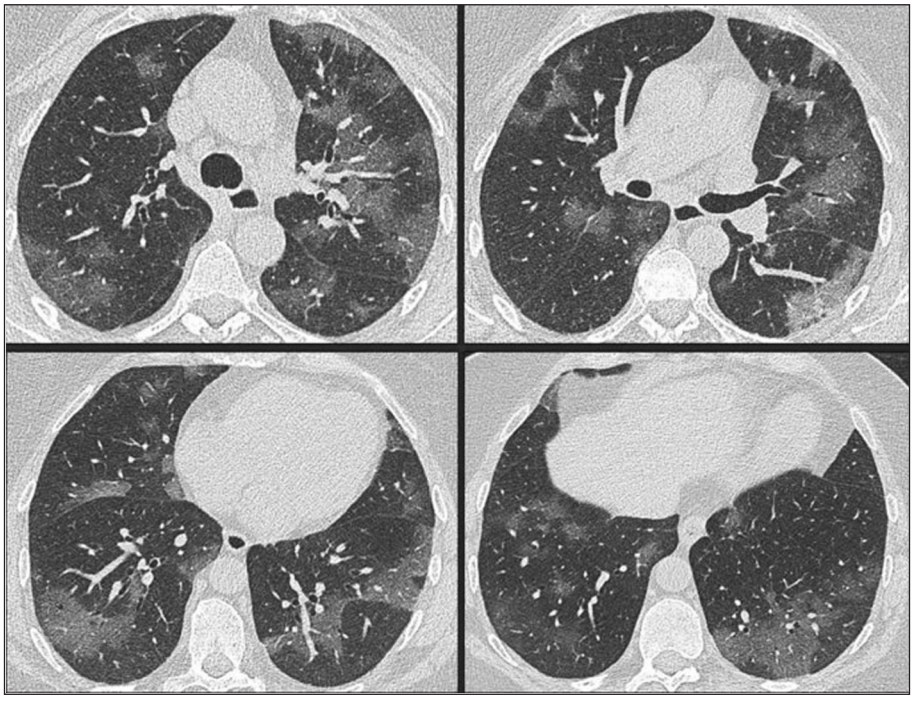


FIGURE 7: CT examination with typical Covid pneumonia findings. It presents with triangular patchy peripheral involvement in a ground glass pattern in both lungs.

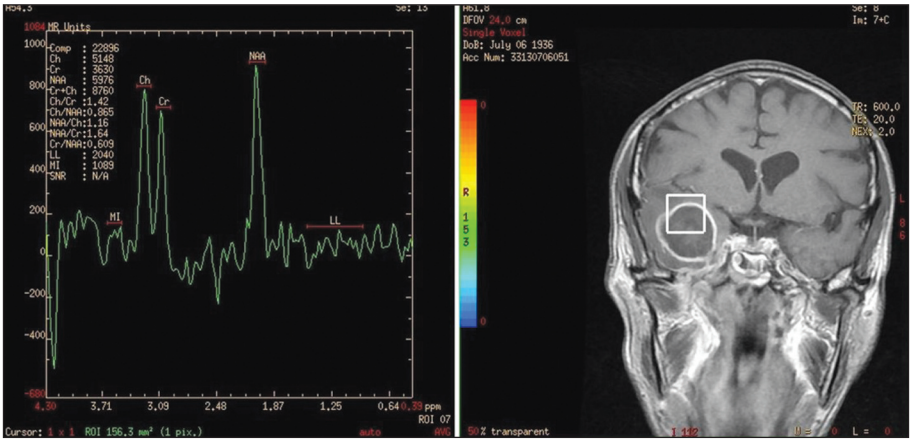


FIGURE 8: In the MR spectroscopy examination, lactate, alanine, cytosolic acid and acetate values are seen to be increased in the abscess cavity.

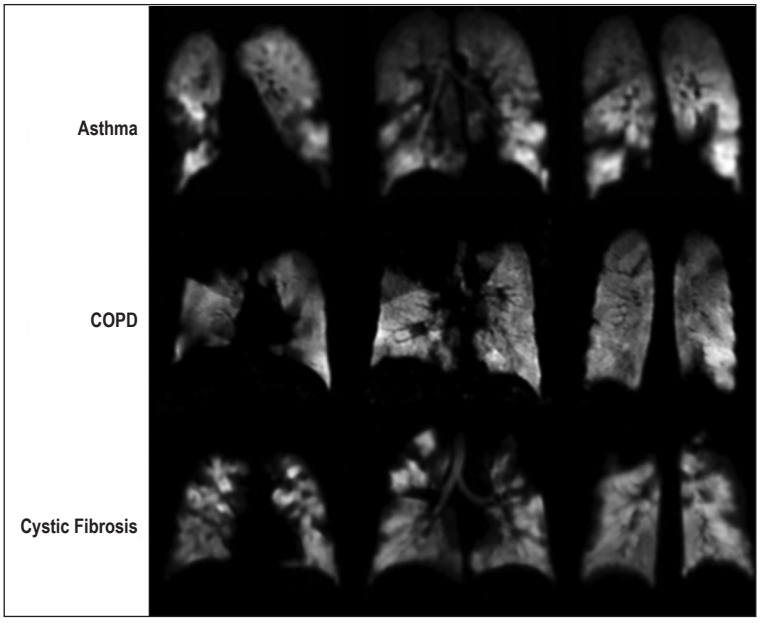


FIGURE 9: Arthritis model in rat experiments. After injection of hyperpolarized contrast, 13C metabolic imaging was performed. The infected subject showed a 65% increase in lactate signal, while alanine remained low.

cases.[13] The same metabolite has also been found useful for detecting inflammation in myocardial infarction.[14] The dynamics of hyperpolarized gases such as asthma and cystic fibrosis have shown that they can be useful in the differential diagnosis of different pathologies (Figure 9).[15] However, these studies have remained on the experimental level and have not been put into daily routine.

In summary, events that develop at the molecular level in inflammation can be demonstrated by different techniques of radiological imaging. In this regard, MR imaging has a high sensitivity due to its working principle. In the near future, it should be expected that diagnostic methods with high specificity will be developed, especially through new metabolites.

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New Approaches to Anti-Inflammatory Therapy: Nanotechnology Applications

Anti-İnflamatuar Tedavide Yeni Yaklaşımlar: Nanoteknoloji Uygulamaları

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ABSTRACT Currently, therapeutic strategies used in anti-inflammatory therapy may not be sufficient to achieve optimal pharmacological activity. For this reason, nanotechnology applications in treatment have been under focus. The biologically compatible nanocarriers should have the appropriate shape, size, and surface charge, and must be loaded with active substance at the appropriate concentration. These nanoparticles allow the controlled drug release, can be aimed to target the disease site, reduce toxicity, and side effects without damaging the surrounding cells and tissues. Among nanotechnology applications, lipidic systems (liposomes) and polymeric systems (nanoparticles) are mostly used. Many active substances are loaded into liposomes and features such as high stability, high loading capacity, active in the inflamed area are obtained. In addition, studies are carried out on the delivery of biotherapeutic large-molecule substances such as nucleic acids, peptides, and proteins in nanoparticle carrier systems. While there are many liposome formulations on the market with clinical research, there are few nanoparticular systems whose clinical studies have been completed due to biocompatibility problems, nanotoxicity and scale-up costs. Studies are ongoing to solve the clinical and industry challenges related to these systems.

Keywords: Anti-inflammatory; inflammation; nanoparticle; liposome; nanotechnology

ÖZET Günümüzde, anti-inflamatuar tedavide kullanılan terapötik stratejiler, optimal bir farmakolojik aktivite elde etmek için yeterli olmayabilir. Bu nedenle tedavide nanoteknoloji uygulamaları ile gündeme gelmiştir. Biyolojik olarak uyumlu nano taşıyıcılar uygun şekle, boyuta ve yüzey yüküne sahip olmalı ve uygun konsantrasyonda aktif madde ile yüklenmelidir. Bu şekilde hazırlanmış nanopartiküller, kontrollü ilaç salımına izin verir, hastalık bölgesini hedefler, çevredeki hücre ve dokulara zarar vermeden toksisiteyi ve yan etkileri azaltabilir. Nanoteknoloji uygulamaları içinde, en çok lipidik sistemler (lipozomlar) ve polimerik sistemler (nanopartiküller) kullanılmaktadır. Birçok aktif madde lipozomlara yüklenerek yüksek stabilite, yüksek yükleme kapasitesi, inflamasyonlu bölgede aktif olma gibi özellikler sağlanmıştır. Ayrıca nükleik asit, peptit ve protein gibi biyoterapötik büyük moleküllü maddelerin nanopartiküler taşıyıcı sistemler içerisinde verilmesi üzerinde çalışmalar yapılmaktadır. Hem piyasada olan hem de klinik araştırması bulunan pek çok lipozom formülasyonu varken, biyouyumluluk sorunları, nanotoksisite ve ölçek büyütme maliyetleri gibi nedenlerle klinik çalışması tamamlanmış nanopartiküler sistem az sayıdadır. Bu sistemlerle ilgili klinikte ve endüstride karşılaşılan zorlukları çözmek için çalışmalar devam etmektedir.

Anahtar Kelimeler: Anti-inflamatuar; inflamasyon; nanopartikül; lipozom; nanoteknoloji

Inflammation represents a mechanism of the body's defence, originating from multiple causes such as infectious agents (viruses, bacteria), physical agents, reactive oxygen species (ROS) and metabolic stress. Inflammation plays an essential role in the healing process, and it is an important part of defence mechanism. A natural defence mechanism is immediately activated in the direction of repair when the human body is damaged. The factors that start the defence mechanism can be internal or external. These factors stimulate the body to increase inflammation mar-

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kers. If the inflammation is uncontrolled, the body can damage its own cells or tissues. This phenomenon leads to many disorders such as autoimmune diseases.[1,2]

The inflammation can also be acute or chronic. The acute inflammation occurs immediately and has five key signs such as pain, redness, loss of function, swelling and heat. However, in some cases acute inflammation progresses without symptoms and just cause tiredness and being unwell. Symptoms of acute inflammation may continue for few days while chronic inflammation may last for months or years. Chronic inflammation may link to various diseases such as diabetes, allergies, psoriasis, and rheumatoid arthritis.[3,4]

When an inflammation occurs the plasma proteins accumulate in the tissue and lead to swelling. This reaction starts healing prosses and neutrophils or leukocyte are released by the body. Generally, inflammation plays a role in the healing prosses, but inflammation may also increase the risk of various diseases, including some cancers, rheumatoid arthritis, atherosclerosis, periodontitis, hay fever and can cause pain. Therefore, the inflammation should be treated by an anti-inflammatory therapy. There are different kinds of therapies including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids and herbal drugs. All these therapies have some advantages and disadvantages. Currently, therapeutic strategies used in anti-inflammatory therapy are mostly insufficient to achieve optimal pharmacological treatment. Drug limitations can be summarized as non-specific biodistribution, low bioavailability and/or short half-life, or the need for high doses for expected effects leading to severe side effects.

In order to avoid some of these limitations, nanotechnology applications in treatment have come to the fore. The biologically compatible nanocarriers must have the appropriate shape, size and surface charge, and must be loaded with active substance at the appropriate concentration. These nanoparticles allow the controlled drug release, can be aimed to target the disease site, reduce toxicity and side effects without damaging the surrounding cells and tissues.

Steroid-based dosage forms have an important place in the treatment of inflammatory diseases or diseases with an inflammatory basis. Corticosteroids are a class of steroid hormones with a wide spectrum, especially glucocorticoids such as hydrocortisone and its derivatives, prednisolone, dexamethasone are frequently used. Nano-formulations of corticosteroids have been prepared due to serious side effects that limit their use. For example, liposome formulations of triamcinolone and its derivatives, which is used as anti-inflammatory and immunosuppressive agent, have been prepared and their anti-inflammatory effect has been increased due to increased localization and absorption.[5]

NANOTECHNOLOGY APPLICATIONS OF INFLAMMATORY DISEASES

Chronic inflammatory diseases are inflammatory bowel disease, osteoarthritis, rheumatoid arthritis and many skin diseases.[6] NSAIDs or glucocorticoids are the first choice when treating the chronic inflammatory diseases.[7-9] Acute diseases may be caused by trauma, airway inflammation, and acute ischemic inflammation and they are treated with high doses of anti-inflammatory drugs.[10]

Different kinds of delivery systems were developed to deliver active substances for topical or systemic application. Due to the side effects of conventional dosage forms studies focus on to improve targeted delivery system for anti-inflammatory therapy. In this direction, nanotechnology has been used and new drug delivery systems (NDDS) were developed for the delivery of anti-inflammatory drugs. In this review, primarily there is a focus on NDDS, especially nano ones, as anti-inflammatory delivery systems.[11]

Nanotechnology covers nano-sized particles applied in different disciplines.[12] Nanomaterials are defined as a material with particle size 1-1000 nm. Since they are designed at small size they can move easily in the human body. They have many advantages due to their structural, chemical, mechanical, electrical and biological properties.[13] The main advantage of these systems is the encapsulation of active substances in the nanoparticles and protection from chemical, physical and biological degradation. They can deliver the active agent more precisely to the target tissues and by a controlled release without damaging other tissues.[14]

Recently new technologies including nanorobots, nanosensors, exosome and liposomes were studied mainly for diagnosis and delivery. Lipid systems like liposomes and micelles including gold or magnetic nanoparticles are now approved by FDA as a new generation-based therapy.

Different kinds of delivery systems are used such as lipidic and polymeric systems. The biocompatible nanomedicines with highly controlled shape, size and surface charge are allowing more efficient treatment options and help to overcome the limitation of conventional treatments.[15] The nanomedicines can control the release of anti-inflammatory agent at the site of application or at the targeted tissue and reduce toxicity and side-effects.[16] Therefore, many nanomedicines were developed to treat di-

seases with an inflammatory background. In this article lipidic systems (exosome, solid lipid nanoparticles, nano lipid carriers, niosome, ethosome and transfersome) and polymeric particles will be reviewed.

LIPID-BASED NANOCARRIERS

Lipid-based delivery systems include liposomes, exosomes, solid lipid nanoparticles (SLN), nano lipid carriers (NLC), niosomes, ethosomes, transfersomes, etc. All these systems have numerous noted advantages and therefore they have received increasing attention in application of inflammatory diseases.

The steroidal drugs generally have low water solubility. Therefore, lipid-based systems serve as a good carrier option for steroidal drugs. Lipidic formulations can sustain the drug release and improve the penetration of molecules through the biological membranes. Non-steroidal drugs encapsulated by lipid vesicles can be more effective than conventional formulations. The general advantages and disadvantages of lipid-base formulations are reported in Table 1.

Liposomes

Liposomes are being used as drug delivery systems since many years. There are some liposomal drugs on the market especially for cancer treatment. Liposomes are small lipidic vesicles consisting of phospholipids. Phospholipid is component of biological membrane. Due to this property, they have low toxicity and low antigenicity. Liposomes are biodegradable and can be metabolized *in vivo* and improve permeation of active substances. The controlled and sustained release can be obtained with the liposomal formulations. Their properties can be controlled by their size, surface charge and composition.[17,18] Despite all the listed advantages, liposomes have some disadvantages which make them unfeasible for pharmaceutical industry. They are in liquid form that makes them risky in point of stability. Liposomes are also not stable under many sterilization methods. Heating damages their structure that means heating and autoclaving sterilization cannot be applied on liposomes. Sterile filtration can be used but filtration reduces the particle sizes.[19] Although the sterilization and stability are the main concerns of liposomes, they are the most preferred nano-systems among lipid-based systems. Phospholipids constitute the basic structure of the most widely used liposomes. They are spherical vesicles formed by one or more biological membrane-like lipid bilayers with an aqueous phase between them and can be easily produced artificially. Drugs such as PEGylated prednisolone liposomes, cyclosporine liposomes, glutathione PEGylated liposomes, methyl prednisolone liposomes have come to the fore with their features such as high stability, high loading capacity, and activation in the inflamed area.

Toita et al. studied on IL-10 loaded phosphatidylserine-containing liposomes. The nano-sized system was tested on a mouse model of obesity. It was shown that liposomes had better macrophage targeting with combination of liposome and IL-10. The authors concluded that this synergistic effect could be used for inflammation related diseases.[20]

Another study was performed to show prednisolone loaded liposome activity. The formulations were tested on the rat model of renal ischemia-perfusion injury. The result showed that the accumulation in the inflamed kidney and the presence of anti-inflammatory macrophages were increased.[21] The anti-inflammatory effect of Cyclosporin A was tested by loading into PEGylated liposomes. The study was performed on the rats, and the significant recovery of the infarct size, brain oedema, and neurological activities compared to free drug were shown.

There are many liposomal systems under clinical trials. Some of the examples are prednisolone loaded PEGylated liposomes, cyclosporine loaded liposomes for treatment of psoriasis and methyl prednisolone loaded glutathione-PEGylated liposomes.[15]

Triamcinolone acetonide is a synthetic glucocorticoid that has anti-inflammatory activity. The liposomal form of

TABLE 1: Advantages and disadvantages of lipid-based systems.

Advantage	Disadvantage
High encapsulation efficient of lipophilic compounds	Initial burst release effect
High permeation through biological membranes	Low encapsulation of hydrophilic drugs
Improved pharmacokinetic properties	Toxicity of lipid substances
Sustained and controlled release	Leakage of molecules from particles
Good stability and biocompatibility	High production cost
Active targeting of molecules and decreasing side effect	

active agent was prepared to increase accumulation in the skin layers and drug stability. The film form of liposomal suspensions was also prepared to make easy topical application. It was concluded that controlled drug release was obtained, and formulations could be a promising system.[22]

Many particulate systems have been used for the treatment of inflammatory diseases. However, liposomes are the most preferred systems due to their biocompatibility, clinical translation, and FDA approval.

Exosomes

Exosomes are liposomal systems specially designed for cellular targeting of biological materials. Normally, many cells secrete exosomes, which are immune system regulators, depending on the cell type. Their activities also vary depending on the cell culture medium in which they are released.[23] Exosomes are cell-free therapy therefore they have lower toxicity and less immune response. Due to these characteristics, exosomes have become very popular drug delivery systems, recently. Many scientific studies are being carried out to study the cellular transport of biological material by exosomes mainly for abnormal immune regulation. The main advantage of exosomes is protecting biological materials from cellular degradation.

It was known that exosomes have many cellular effects in many diseases. In addition to their natural effect, they are also good options for delivery of drugs to the targeting site. Exosomes have structural stability and drugs can be loaded by physical, chemical, and biological approaches.[24] The lipidic bilayer of vesicles protect them from enzymatic degradation and minimize the loss of active substances.

The drugs can be administrated via several routs. Exosomes also can be applied by intranasal, intracranial, intraperitoneal, and intravenous routes. Their shape and structure help to pass through the blood-brain barrier that is very important advantage for brain targeting. Exosomes have low immunogenic response in comparison to other nanocarriers such as polymers, dendrimers, and liposomes. Their natural structure also decrease toxicity.

The research show that exosomes have many advantages as drug delivery systems. For example, curcumin loaded mouse lymphoma ceel-derived exosomes enhanced apoptosis of CD11b+ Gr-1+ myeloid cells and activated anti-inflammatory response.[25] In another study, a collagen induced arthritis was formed in mouse model and it was seen that exosomes reduced joint inflammation. The acute and chronic neuroinflammations are important factors in pathogenesis of neurodegenerative diseases. Exosomes used in neurogenerative diseases are focused on inflammation therapy. The mesenchymal stem cell-derived exosomes showed immunomodulatory and neuro-regenerative effects in status epilepticus. Exosomes from intranasal mesenchymal stem cells alleviated inflammation and inhibited abnormal neurogenesis in a mouse model of status epilepticus. The exosomes were also used for allergic airway inflammation in a mouse model. It was seen that number of eosinophils decreased dramatically and the number of neutrophils remained unchanged in comparison with untreated mice. Cytokines such as IL-4, IL-5 and IL-3 were significantly reduced in bronchoalveolar lavage fluid which contribute to allergic responses.[26] Chronic skin diseases may occur as a result of immune system responses in the skin.[27] Atopic dermatitis is one of the skin immune system diseases. Cytokines such as IL-4, IL-31 and TNF-α, IgE and eosinophils levels were decreased by anti-inflammatory effect of exosomes from human adipocyte mesenchymal stem cell.

As a conclusion there are many studies that show the anti-inflammatory effect of exosomes. Nowadays, studies are focusing on standardization of processing methods and minimizing differences between each batch and safety of systems.

Solid Lipid Nanoparticles (SLNs)/Nano Lipid Carriers (NLCs)

Solid lipid nanoparticles are first generation of lipid nanoparticles. The composition of the system is similar to oil-in-water nanoemulsions. But SLNs consist of solid lipid instead of liquid lipid that used in nanoemulsions and have more physical stability than nanoemulsions. The solid structure is formed by a rigid core which is surrounded by surfactant layer. Other advantages of system are low surfactant concentration, low toxicity risk and better biocompatibility. Beside these advantages they have two main limitations: low drug loading capacity and drug leakage during storage.

NLC systems were developed to overcome the limitations of SLNs.[28] NLCs are consisting of solid and liquid lipids together to form a lipid core. Thus, they have lower melting point as compared to SLNs, but they are in solid state at human body temperature.[29] Addition of liquid lipid provides increase in molecular disorganization, and this prevents molecules from leakage during storage.

SLNs and NLCs exhibit excellent biocompatibility and biodegradability due to their components. All components of systems use generally recognized as safe (GRAS).

Their production is generally solvent-free and they can be sterilized by the autoclave. Therefore, they can be easily applied to industry.

SLN and NLC formulations are manly used for anti-inflammatory treatment of ocular diseases. NSAIDs are encapsulated by these lipid-based vesicles. For example, diclofenac sodium was loaded SLNs, ibuprofen was loaded NLCs for analgesic effect and cyclosporine A-loaded SLN formulations were prepared for ocular anti-inflammatory diseases. The addition of polymers to these lipidic systems can improve duration of formulations at the site of application and improve the effect of active agent. Chitosan is one of the most used polymers, due to the adhesive properties. SLN and NLC systems can also decrease frequency of drug administration and increase the patient compliance.[30]

SLNs of dexamethasone and butyrate was used for arthritis. The results of *in vivo* study showed that after oral administration to mice, significant decrease in pro-inflammatory cytokines was observed. They were found more effective at doses 10 times lower than with free drugs.[31] In another study hyaluronic acid coated SLNs were loaded with prednisolone, and it was shown that a selective accumulation in inflamed tissue could be observed after injection to mice. A good therapeutic efficacy based on analysis of joints, pannus formation, bone preservation and reduction pf pro-inflammatory cytokines were reported.[32]

Cyclosporine A loaded solid lipid nanoparticles were prepared for ocular delivery. Compritol 888 ATO, Poloxamer 188 and Tween 80 were used to enhance penetration and investigate the cellular uptake. The results were confirmed by the internalization of vesicles in cornea and in RCE cell lines.[33]

Niosomes

Niosomes are similar to liposomes, but they contain nonionic surfactant instead of phospholipids. They can carry amphiphilic and lipophilic drugs with a wide range of solubility because of their nature. Niosomes can protect active agents from biological environment and chemical and physical degradation. Niosomes are biodegradable, biocompatible and nonimmunogenic due to their composition.[34] The drugs are encapsulated in the niosomal vesicles therefore a controlled and sustained release can be obtained with them. They also improve the clearance of molecule, and a specific targeting can be achieved. Niosomes can improve bioavailability of poorly soluble drugs and can enhance topical application efficacy. Niosomal formulations are studied for many diseases including inflammatory ones. Glycyrrhiza glabra is natural compound that has anti-inflammatory effect in the treatment of dermatitis, eczema and psoriasis. Glycyrrhiza glabra loaded niosomes were investigated for its anti-inflammatory effect. Chemical skin erythema has been created in human and mice. It was shown that the activity was increased by encapsulation.[35] Zingiber cassumunar loaded niosomes had enhanced chemical stability and the skin permeation was increased in comparison with commercial product. For local subcutaneous inflammation therapeutic ultrasound increased anti-inflammatory effect.[36] Resveratrol is a well-known anti-inflammatory natural agent. But it has low water solubility and bioavailability that reduce its potential therapeutic effect. The chemical stability and limited skin permeation can be eliminated by encapsulating in niosome vesicles. It was shown that resveratrol loaded niosomal hydrogel systems could enhance the permeation trough and deposit in the skin. Niosomal hydrogels was applied to a rat paw edema model, and it was seen that the system had prolonged therapeutic action.[37]

Ocular drug application has some limitations such as nasolacrimal drainage and the corneal barrier. Only maximum 5% of applied drugs can reach intraocular site. Flurbiprofen is one of the anti-inflammatory drugs that used for ocular application. Flurbiprofen loaded niosomes was used in albino rabbits and it was observed high ocular bioavailability and fast reducing in inflammation than solution of drugs.[38]

Topical route is an alternative route to oral administration when drugs have side effects. Serratiopeptidase has systemic side effects when applied by oral route. The molecule has therapeutic effect on arthritis, fibrocystic breast disease, chronic bronchitis, and carpal tunnel syndrome. Niosomal topical formulation of active substance showed improved local effects and reduced adverse effects. The activity was found similar to diclofenac gel.[39]

The anti-inflammatory effect of prednisolone loaded elastic niosomes was tested on rabbits subjected to a clove oil induced ocular inflammation. The formulation was consisted of Span60, cholesterol and ethanol.[15] The stability of compound and ocular tolerability increased, and ocular irritation decreased. A reduction of half of the time for complete healing was observed, in addition to a reduced intraocular pressure, one of the main side effects observed with conventional anti-inflammatory treatments. Besides, this system demonstrated good physical stability for at least 2 months in the refrigerator with good ocular tolerability and minimal ocular irritation, suggesting good potential for translation into the clinic.[40] Niosomal formulations of can-

nabidiol showed an anti-inflammatory effect on carrageenan-induced inflammation at mice. The same results were obtained with curcumin, diclofenac, and meloxicam niosomal formulations.

As a summary, niosomes represent a highly effective delivery system for anti-inflammatory diseases and they are more effective than conventional formulations.

Ethosomes

Ethosomes are a kind of liposomal system that consist of high percentage of ethanol inside. High ethanol concentration provides them ultra-deformable, soft and flexible structure. Due to ethanol content, they can interact with the phospholipids in the skin and improve transdermal and dermal bioavailability of compounds. They can improve the stability of the agent by encapsulation in the vesicles. Ethosomal formulations are mainly used for pain management in skin diseases. Flurbiprofen loaded niosomes showed an increased anti-inflammatory effect and a sustained release of active agent for 24 hours.[41] The encapsulation of active substance in ethosomes protects form environmental conditions such as pH and enzymatic degradation.

Ammonium glycyrrhizinate loaded ethosomes were tested on skin erythema model. The permeation, toxicity and anti-inflammatory effect of formulations were evaluated. The results showed that the ethosomal systems had very good skin tolerability.[42]

A corticosteroid HB17 was loaded in ethosomal vesicles, and the permeation was increased. It was concluded the system had advantages such as increasing permeation, decline inflammation, simple preparation, improved patient convenience and compliance. It was reported there was a synergistic mechanism between ethanol, surfactant, vesicles and skin lipids.[43]

Ethosomes are good alternative as transdermal drug delivery systems due to the penetration enhancer effect. But they still have some problem with stability of vesicles and yield capacity of production.

Transfersomes

Transfersome is a drug delivery system that were introduced in the 1990s. They are composed of phospholipids and an edge activator such as Tween80, Span80 and sodium cholate. The edge activator plays a role as membrane softening agent. They have ultra-deformable properties due to composition. After topical application they can penetrate into deeper layer of skin spontaneously by changing membrane flexibility. Transferosomes can across narrow pores because of extremely deformable structure.[44] The preclinical studies showed their advantages with many drugs in different kinds of diseases. The clinical studies with desired therapeutic agents are under investigation at different stages of clinical trials.

For example, ketoprofen loaded transfersomes were designed and their safety and efficacy for the treatment of osteoarthritis of the knees was tested in phase III clinical study. The study was conducted over a six-treatment periods and the results were compared with placebo. It was concluded that ketoprofen loaded transfersomes had a superior therapeutic potency in relieving the pain and less adverse effect was seen.[45] Triamcinolone acetonide is a corticosteroid that is used to treat the symptoms of topical inflammatory dermatoses, oral inflammatory or ulcerative lesions. It has some adverse effect such as other corticosteroids. A randomized controlled trial was performed to compare triamcinolone acetonide containing cream and ointment with triamcinolone loaded transfersomes. The risk-benefit ratio of vesicular system was investigated, and it was seen that transfersomes improve the risk-benefit ratio of topical triamcinolone, significantly.[46] Another study result with same molecule showed that transfersomes had prolonged effect, increased potency and they reduced the therapeutic dosage.

Capsaicin loaded transfersomes were prepared by thin film hydration method with different composition. The results of *in vivo* studies confirmed that the system showed superior inhibitory activity in reducing arthritis associated inflammation. An enhanced permeation and better tolerance were observed with the transfersomes. It was concluded that Tween80 resulted in the highest entrapment efficiency and small particle size.

Transfersomal gel formulations were prepared to increase the stability of vesicular system and permeability of drug.[47] Aceclofenac loaded transferosomal gels were prepared by film hydration method and consisted of soybean oil, Span80 (edge activator) and Carbopol 934. The *in vitro* drug release study results showed that gelation decrease the release rate of active substance in comparison to transferosome suspension. The permeation through the skin was compared with commercial gel and higher penetration rates was obtained with transferosome gels. Similar results were obtained by curcumin loaded transfersomes. The immunohistochemistry results showed that gel inhibited the action of NF-kB and prevent the occurrence of tissue inflammation by reducing TNF-α and IL-6.

Some other anti-inflammatory agents such as diclofenac sodium, celecoxib, curcumin and mefenamic acid were loaded transfersomes for topical application. The stability and efficacy of the molecules were improved by encapsulating into transfersomes

EMULSIONS (MICROEMULSIONS AND NANOEMULSIONS)

Emulsions can be classified such as macroemulsion, microemulsion, nanoemulsions and multiple emulsions. Macroemulsions and microemulsions have large droplet size. Therefore, they are not reviewed in this chapter. Microemulsions consist of oil, water surfactant and cosurfactant phases. They are homogenous dispersions and can be in water-in-oil or oil-in-water type. They are thermodynamically stable systems and their size vary between 10-400 nm. Microemulsion consists of high concentration of surfactant and cosurfactants that have penetration enhancer effect. Due to this ability, microemulsions are used as transdermal delivery systems for many active agents. Moreover, the main advantages of these systems are the simplicity of the preparation process and the high drug loading capacity. The hydrophilic and lipophilic substances can be loaded to microemulsions' different phases. Microemulsions protect active agents from chemical, physical and biological degradation and a controlled release can be obtained.[11] Microemulsions can be applied via several routes such as transdermal, oral, and parenteral routes.

Origanum vulgare containing microemulsions was prepared for transdermal application and the release of carvacrol was investigated. Tween60 and butylene glycol was used as surfactant and cosurfactant, respectively. The result showed that cutaneous absorption was significantly increased with microemulsion than Origanum vulgare solution. The anti-inflammatory test was performed, and it was seen that the levels of IL-6 were significantly inhibited and TNF-α inhibition was comparable to dexamethasone which is used as a reference drug for anti-inflammatory therapy.

In another study, piroxicam loaded microemulsion was prepared for oral application. It is known that piroxicam has low drug solubility. So, the aim of study was to increase solubility properties and to obtain more bioavailability. The formulation was consisted of Tween80, Cremophor and PEG400 as surfactants that have penetration enhancing effect. The plasma concentration time profiles of microemulsions were evaluated and it was found that the area under the curve was 2.7-fold and Cmax was 4-5-fold higher than control groups.

Lysine clonixante was incorporated to microemulsion for intramuscular administration. Labrafil was used as oil phase and Lauroglycol and Tween80 was used as surfactant. The results showed that a controlled release of active agent was obtained because of Labrafil which promoted droplet size and viscosity increase leading a slower *in vitro* release. Carrageenan-induced paw edema test in *in vivo* study showed that a greater analgesic effect was seen in comparison to the control group.[48]

Nanoemulsions are very similar to microemulsions for their components and preparation methods. The main difference is that the nanoemulsions are thermodynamically metastable while microemulsions are thermodynamically stable.[49] Nanoemulsion formulations are used for the delivery of anti-inflammatory drugs via dermal route. For example, Aceclofenac was formulated in nanoemulsion consisting of Labrafil, Tween80, Transcutol P, Triacetin and water. The results showed that an improved permeation rate and a decrease irritation effect was obtained with nanoemulsions in comparison with aceclofenac gel. In another study, 3,5-dihydroxy-4-isopropylstilbene (DHPS), was integrated into a nanoemulsion for anti-inflammatory activity. It was concluded that the permeability and solubility of drug was enhanced with nanoemulsion components.[50]

It can be concluded from the results of microemulsion and nanoemulsion studies, reducing droplet size, increasing solubility, and enhancing permeation by components, increased the anti-inflammatory activities of drugs.

POLYMERIC NANOPARTICLES SYSTEMS

Nanoparticles are solid colloidal particles with sizes ranging from 10-1000 nm, prepared with polymers of natural or synthetic nature, that release dissolved, trapped, or adsorbed active substance in a controlled manner. In recent years, studies have been carried out on the delivery of active substances with solubility and stability problems. The biotherapeutic large-molecule substances such as nucleic acids, peptides and proteins are also incorporated in nanoparticle drug carrier systems. For this purpose, lipid nanoparticles, which have a low probability of acute and chronic toxicity due to the lipids they contain, have been frequently used.

Polymeric nanoparticles are produced with natural or synthetic polymers. The polymers should be biodegradable and biocompatible. Poly-lactic-acid (PLA) and poly-lactic-co-glycolic acid (PLGA) are mainly used to prepare nanoparticles. It is known that polymeric nanoparticles are

more stable than lipidic nanoparticles. When compared with conventional formulations, solubility of drugs can be modified, and absorption of molecule can be increased. Polymeric nanoparticles can be designed as nanocapsules or nanospheres depend on composition, structural properties, and production method. Nanocapsules are prepared with a polymer shell and an oil core but nanospheres include only polymeric structure.[51] There are many studies that studied polymeric nanoparticles for several diseases. Recently, hybrid nanoparticles have been studied for anti-inflammatory therapy.

PLGA is a comparatively hydrophobic synthetic polymer that FDA has approved. Therefore, in many studies PLGA is used for anti-inflammatory treatment. PLGA nanoparticles can encapsulate different kind of drugs and target them to the site Curcumin has been encapsulated in PLGA nanoparticles for treatment of anti-inflammatory diseases. Glycyrrhizic acid loaded polycationic chitosan nanoparticles was prepared and polyanionic katira gum was added. The system showed anti-inflammatory action and controlled release. The release kinetics was zero-order and sustained the release. The mechanism of the release was diffusion of drug and erosion in the polymeric matrix. It was concluded that encapsulation has increased the anti-inflammatory effect by improving bioavailability of drug.[52] In another study, celecoxib that is a non-steroidal anti-inflammatory drug was formulated with γ-cyclodextrin and randomly methylated β-cyclodextrin to improve solubility of drug. These nanoaggregates were combined with mucoadhesive polymers such as hydroxypropyl methylcellulose or hyaluronic acid. The result showed that eye-drop formulation improved drug permeation by increasing duration at the site of application.[53]

In a study, diflucortolone valerate was formulated with lecithin/chitosan nanoparticles to increase localization of drug in the skin layers. The high entrapment efficiency and sustained release of drug without any initial burst release was obtained with the formulations. The nanoparticles were incorporated in chitosan gel for topical application. *in vivo* studies revealed higher edema inhibition with nanoparticles in comparison to commercial cream.[54]

The combination of two therapeutic anti-inflammatory drugs that have different mechanism of action was prepared as particular systems. The cationic dexamethasone palmitate nanoparticles were used to with DOTAP. The combination system was evaluated for cellular uptake by flow cytometry and confocal microscopy on RAW264.7 macrophages. TNF-α inhibition was determined on LPS-activated macrophages and a larger inhibition was observed. It was concluded that this combination system could be a novel strategy to cure inflammatory diseases.[55]

CONCLUSION

This review focuses on new nanocarrier systems that are used in studies to evaluate their effectiveness in inflammatory diseases. It has been observed by many research that nanoscale drug delivery systems can successfully transport anti-inflammatory agents to the site of targeting in a controlled manner. They can optimize drug safety and efficacy by improving biodistribution and pharmacokinetics properties.

However, while there are many liposome formulations on the market with clinical research, there are few nanoparticular systems whose clinical studies have been completed due to biocompatibility problems, nanotoxicity and scale-up costs.

In the future, it is necessary to solve and optimize the difficulties encountered so that these systems can be produced safely, biocompatible, biodistributed in appropriate quantities and reproducibly by designing industry standard scale-up processes.

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Inflammation and Immunity

İnflamasyon ve Bağışıklık

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ABSTRACT Inflammation is not a singular phenomenon, but rather a combination of a wide variety of immune responses to a particular stimulus (such as pathogens, tissue damage). The inflammation process begins with the recognition of pathogens, tissue damage and foreign substances to the host by the cells of the innate immune system through pattern-recognizing receptors (PRR). As a result, various proinflammatory cytokines and chemokines are produced and released to perform the effector functions of inflammation. Blood-borne neutrophils and monocytes migrate to the scene by extravasation with the activation of endothelium and the effect of chemokines. Neutrophils release toxic compounds such as reactive oxygen species, reactive nitrogen species, and various proteases, which have the potential to harm both the pathogen and the host. At this stage, macrophages and dendritic cells process antigens and present to T cells. The innate immune response and the characteristic of the resulting inflammation are factors that determine which direction the adaptive immune response will polarize. Resolution of inflammation is accomplished by promoting the conversion of leukotrienes produced by neutrophils, macrophages, and other cells into lipoxins, which initiates the cessation of inflammation.

Keywords: Inflammation; immunity; inflammasome; PAMP; DAMP

ÖZET İnflamasyon tekil bir olgu değil, aksine belli bir uyarana (patojenler, doku hasarı gibi) karşı oluşturulmuş çok çeşitli bağışıklık yanıtlarının bileşimidir. Patojenler, doku hasarı ve konağa yabancı maddelerin doğal bağışıklık sisteminin hücreleri tarafından patern tanıyan reseptörler (PRR) aracılığıyla tanınması ile inflamasyon süreci başlar. Bunun sonucunda, çeşitli proinflamatuvar sitokinler ve kemokinler üretilerek inflamasyonun efektör işlevlerini gerçekleştirmek üzere ortama bırakılır. Kan yoluyla taşınan nötrofiller ve monositler endotelin aktivasyonu ile ve kemokinlerin etkisiyle damar dışına çıkarak olay bölgesine göç eder. Nötrofiller hem patojene hem de konağa zarar verme potansiyeline sahip olan reaktif oksijen türleri, reaktif nitrojen türleri ve çeşitli proteazlar gibi toksik bileşikleri olay yerine bırakır. Bu aşamada, makrofajlar ve dendritik hücreler, antijenleri alıp T hücrelerine sunulacak şekilde işler. Doğal bağışıklık yanıtı ve oluşan inflamasyonun karakteri edinilmiş bağışıklık yanıtın ne yöne doğru kutuplaşacağını belirleyen faktördür. İnflamasyonun çözülmesi, nötrofiller, makrofajlar ve diğer hücreler tarafından üretilen lökotrienlerin inflamasyonun sonlanmasını başlatan lipoksinlere dönüşümünü teşvik etmesiyle başlar.

Anahtar Kelimeler: İnflamasyon; bağışıklık; inflamazom; PAMP; DAMP

Inflammation is not a singular phenomenon, but rather a combination of a wide variety of immune responses to a particular stimulus. It is a challenge where the immune system uses every weapon in its arsenal. Therefore, inflammation is defined as a protective immune response of the organism to stimulation by invading pathogens and other factors such as damaged cells, toxic compounds, or irradiation, and results in removal of initial injurious stimuli, the clearance of damaged and necrotic cells and tissue repair and remodelling.[1,2] Almost all inflammatory response processes share a common mechanism, which can be summarized as follows: 1) recognition of detrimental stimuli by cell surface pattern receptors or intracellular sensors; 2) activation of inflammatory pathways; 3) release of inflammatory mediators; 4) recruitment of inflammatory cells; 5) resolution of inflammation.[2]

KAYNAK GÖSTERMEK İÇİN:
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RECOGNITION OF DETRIMENTAL STIMULI BY CELL PATTERN RECEPTORS

Inflammatory response is initiated by the recognition of evolutionarily conserved structures on pathogens (pathogen-associated molecular patterns, PAMP) or endogenous stress or danger signals (damage-associated molecular patterns, DAMP), also known as alarmins, through germline-encoded pattern-recognition receptors (PRRs) mainly expressed by immune cells such as monocytes, macrophages, neutrophils and dendritic cells, lymphocytes as well as fibroblasts and epithelial cells (Table 1).[3,4] Recognition of pathogen-derived products such as lipopolysaccharide (LPS), flagellin, RNA and CpG DNA or occurs by cell-associated PRRs that are located either at the surface or within the cell, Host cell-derived danger signals such as heat shock proteins, uric acid crystals, high-mobility group box 1, serum amyloid A, and products of purine metabolism released from damaged or stressed cells are sensed by the receptors such as TLRs, intracellular nucleotide binding domain and leucine-rich-repeat containing receptors (NOD-like receptors or NLRs),and purinergic receptors.[3-5]

Cell-asscoiated PRRs are composed of the Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), NOD-like receptors (NLRs), CDSs, and Scavenger reseptors. Pathogen-derived products such as lipopolysaccharide (LPS), flagellin, nucleic acids are recognized by TLRs either at the surface of the cell or within endoplasmic vesicles (Table 2).[4] Host cell-derived danger signals or alarmins are sensed by DAMP receptors such as receptor for advanced glycation endproducts (RAGE), TLRs, and purinergic receptors.[5,6] Recognition of PAMPs and DAMPs by these PRRs leads to the activation of signaling pathways that ultimately causes the expression of the transcription factors including nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and interferon regulatory factors (IRFs).[5,7] These transcription factors control the expression of many inflammatory response genes, including TNF-α, IL-1β.[5]

ACTIVATION OF INFLAMMATORY PROCESSES

Inflammatory processes involve common inflammatory mediators and regulatory pathways. Cellular stimuli activate intracellular signaling pathways, and therefore initiate inflammatory processes through the release of proinflammatory mediators such as cytokines and chemokines.[2,8] For instance, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) lead to the local activation of macrophages and neutrophils by autocrine and paracrine effects. These cytokines can also cause endocrine (systemic) effects, such as induction of production of acute-phase proteins, platelet activation, fever, fatigue and anorexia, when they are released in large amounts.[8] Microbial products, alarmins and cytokines mediate inflammation through interaction with their respective receptors, such as TLRs, NLRs, and various cytokine receptors.[9] Receptor activation initiates important intracellular signaling pathways, including nuclear factor kappa-B (NF-κB), the

TABLE 1: Pathogen-associated molecular patterns and damage-associated molecular patterns.

	Molecular Patterns	
Pathogen-associated molecular patterns, PAMP		**Pathogen type**
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG DNA	Virus, Bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fıngi, bacteria
	Glucan	Fungi
Damage-associated molecular patterns, DAMP		
Stress-induced proteins	Heat shock proteins (HSPs)	
Crystals	Monosodium urate	
Proteolytically celeaved extracellular matirx	Proteoglycan peptides	
Mitochondria and its components	Formylated peptides and ATP	
Nuclear proteins	High mobility group box protein-1 (HMG-1), histones	

TABLE 2: Pattern recognition molecules.

Pattern Recognition Receptor	Location	Specific example	Ligands
Cell-associated			
TLRs	Plasma membranes or endosomal membranes of Denrditic cells, phagocytes, B cells, endothelial cells and many other cells	TLR1-9	Various microbial moleculse such as viral nucleic acids, bacterial LPS and petidoglicans, flagellins
CLRs	Plasma membranes of phagocytes	Mannose receptor	Microbial surface carbohydrates
		DC-sign	
		Dectin-1 and -2	Glucan, especially present in fungal cell wall
NLRs	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2	Bacterial cell wall peptidoglycan
		NLR Family (inflammasomes)	Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs	Cytosol of phagocytes, and other cells	RIG-1, MDA-5	Viral RNA
CDSs	Cytosol of various cells	AIM-2, STING-associates CDSs	Bacterial and viral DNA
Scavenger Receptors	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe recptors	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing N-Formylmethionyl residues
Soluble			
Pentraxins	Plasma	C-reactive protein	Microbial phosphroylcholine and phosphatydilethanolamine
Collectins	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fructose
	Alveoli	Surfactant proteins (SP-A and SP-D)	Various microbial structures
Ficolins	Plasma	Ficolin	N-acetylglucosamine and lipoteichoic acid componenets of Gram-positive bacteria
Complement	Plasma	Various complement components	Microbial surfaces

mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways, as well as inflammasomes.[2,10] Induction of these pathways leads to the expression of several immune response genes such as cytokines, inflamatory proteins and enzymes.[11-13]

The NF-κB is one the most important transcription factor which plays important roles in many inflammatory and immune responses.[2] Under physiological conditions, IκB proteins present in the cytoplasm together with NF-κB and inhibits its translocation to nucleus.[14] PRR induction initiates signal transduction mechanisms to activate IκB kinase (IKK), composed of IKKα and IKKβ subunits, and a regulatory IKKγ subunit. IKK leads to NF-κB activation through IκB phosphorylation, which results in its degradation by the proteasome and the subsequent release of NF-κB for nuclear translocation and gene transcription activation (Figure 1).[14,15]

MAPKs are a family of serine/threonine protein kinases which include extracellular-signal-regulated kinase ERK1/2, p38 MAP Kinase, and c-Jun N-terminal kinases (JNK).[16] Erk1/2 is activated by MKK1/2, after activated by Raf, whereas JNK is activated by MKK4/7, folowing the activation by MEKK1/4, ASK1, and MLK3. In the p38 pathway, MKK3/6, which is activated by MLK3, TAK, and DLK, activates p38 MAP kinase. Activated MAPKs phosphorylate various proteins, including transcription factors, and therefore, leads to the regulation of inflammatory responses.[17]

The JAK-STAT pathway is a signaling mechanism through which extracellular factors can control gene expression, and which involves diverse cytokines, growth factors, interferons, and related molecules, such as leptin and growth hormone.[18] Receptor-associated JAKs are activated by ligands and activate STATs, which are cytoplasmic transcrip-

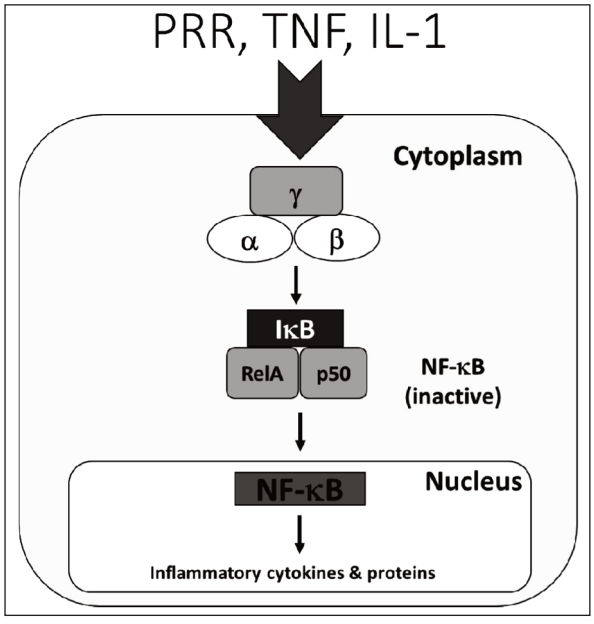


FIGURE 1: NF-κB pathway is triggered by TLRs and inflammatory cytokines causing the activation of RelA/p50 complexes (active NF-κB).

tion factors. Cytoplasmic STATs undergo subsequent dimerization before translocation to the nucleus.[19] STAT proteins translocated into the nucleus anda dephosphorylated bind target gene promoter regions to activate transcription of inflammatory genes.[20]

The inflammasomes are the receptors and sensors of the innate immune system that induce inflammation in response to PAMPs and DAMPs. Inflammasomes lead to the cleavage of the IL-1 family of cytokines and lead to an inflammatory form of cell death termed pyroptosis.[21] The main structure of inflammasome complexes is composed of a receptor or sensor from the nucleotide-binding domain and a leucine-rich repeat containing protein (NLR), the absent in melanoma 2 (AIM2)-like receptor, tripartite motif (TRIM) proteins, an adaptor protein called apoptosis-associated speck like protein containing caspase recruitment domain (ASC), and the cysteine protease Caspase-1. Activation of the inflammasome sensors trigger ASC and CASPASE-1 via homotypic interaction of the CARD domain.[22] CASPASE-1-mediated cleavage then facilitates the generation of mature the proforms of IL-1β and IL-18. Both IL-1 and IL-18, in their mature forms, are potent proinflammatory cytokines (Figure 2).[5] Inflammassome activation also leads to alternative outcomes such as phagosome maturation, autophagy, glycolysis, lipid metabolism, and oxidation of arachidonic acid to generate eicosanoid signaling molecules, as well as inflammatory pyroptotic cell death.[5]

RELEASE OF INFLAMMATORY MEDIATORS

Activation of inflammatory cells by vaious stimuli induce production of inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and inflammatory proteins and enzymes.[2]

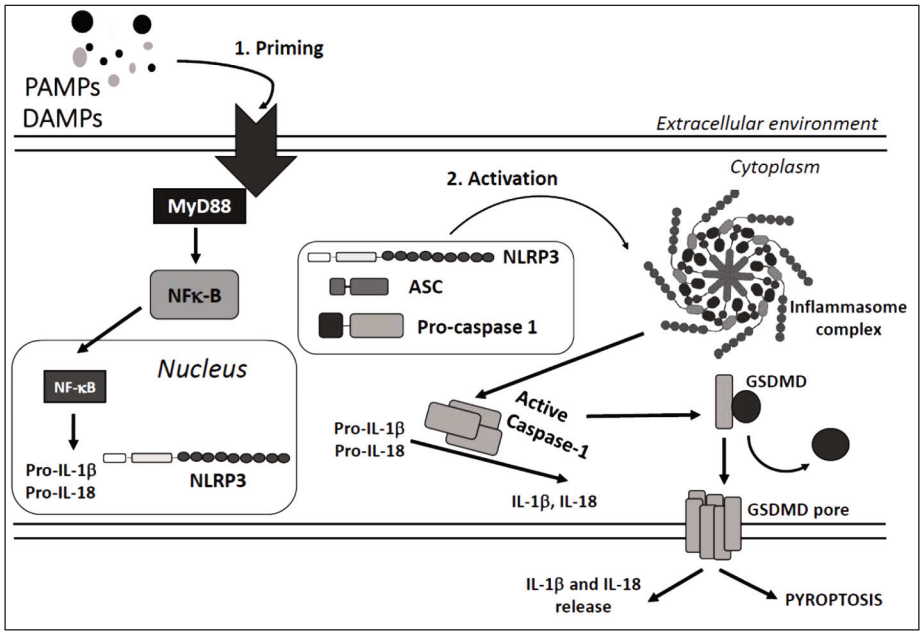


FIGURE 2: Inflammasome activation pathway. PAMP and DAMP signals are recognized by PRRs, which then cause the activation of caspases. Active caspase-1 will promote the IL-β and IL-18 release or pyroptosis, through GSDMD cleavage and pore formation.

Cytokines not only modulate the immune response to infection or inflammation but also regulate inflammation itself via various network interactions. On the other hand, exaggregated production of proinflammatory cytokines can cause tissue damage, hemodynamic changes, organ failure, and ultimately death.[2,8] Cytokines, such as TNF-α and IL-1β, stimulate endothelial cell activation and increase vascular permeability. This facilitates entrance of immune cells into tissues at the site of infection. They can also lead to capillary leakage, vasodilation and hypotension.[23] The cytokine IFN-γ, produced by T helper 1 (Th1) cells activates neutrophils, whereas the cytokine IL-22, derived from Th17 cells and innate lymphoid cells (ILCs), acts on epithelial cells and subsequently stimulates the synthesis and release of antimicrobial peptides, such as defensins.[24]

Inflammatory proteins, apart from cytokines, including C-reactive protein (CRP), haptoglobin, serum amyloid A, fibrinogen, and alpha 1-acid glycoprotein, support providing homeostasis and reducing microbial growth during trauma, stress, or infection. Abnormal activation of certain enzymes, including high-mobility group box 1 (HMGB1), superoxide dismutase (SOD), glutathione peroxidase (GPx), NADPH oxidase (NOX), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, may be involved in the development of inflammatoryy conditions, such as cardiovascular disease and cancer.[2]

Inflammatory proteins and enzymes have been used as biomarkers for the diagnosis and follow-up of inflammation, infection, and trauma in medicine.

RECRUITMENT OF INFLAMMATORY CELLS

Once inflammatory reaction has started, the first leukocytes recruited to the site of infection or injury are neutrophils. These are followed by inflammatoyry monocytes, lymphocytes (natural killer cells [NK cells], T cells, and B cells), and mast cells.[2] Monocytes can produce some proinflammatory cytokines, but they differ from macrophages by not expressing the adhesion G-coupled receptor E1, also called F4/80. Monocytes are also be able to differentiate macrophages and dendritic cells in the tissues, depending on signals that they receive from their environment. Neutrophils are the first cells that arrived in infected or injured tissue, responsible for high microbiocidal activity, whilst monocytes/ macrophages and dendritic cells are recruited later on, and they play roles in the initiation of adaptive immune response through antigen processing and presentation.[25] Neutrophils digest and try to eliminate the pathogen or the injurious agent, by phagocytosis and the production of ROS, proteases, and neutrophil extracellular traps (NETs).[26] NET is a distinct cell death program, in which nuclear chromatin coated with histones, elastase, myeloperoxidase (MPO), and cathepsin G is released into the extracellular space and lyse the bacteria.[27,28] Also, NET formation can occur independent of cell death in non-infectious stimuli (Non-lytic NET), and this is mitochondrial and not nuclear, lacking histones. This appears more rapidly in 5-60 min in comparison to 3-5 h for the lytic NET release.[29] MPO is mainly produced by neutrophils fluid after oxidative stress and inflammatory responses, and its activity involves the production of ROS.[30] Controlled MPO release at the site of infection is crucial, and abnormal release or function is associated to different diseases expressing chronic inflammation. Tissue-resident macrophages also play roles in resolution of inflammation and tissue repair.[31] They may be in the "M1" pro-inflammatory phenotype or the "M2" antiinflammatory phenotype.[32] M1 macrophages are mainly active in case of infection, and express inducible nitric oxide synthetase (iNOS) and CD40, produce proinflammatory cytokines such as IL-6 and TNF-α. M2 macrophages express arginase I and CD206 and produce TGF-β and IL-10 to start tissue repair and re-modelling.[25] Macrophages and dendritic cells are processed so that antigens are taken and presented to T cells. The innate immune response and the character of the resulting inflammation are factors that determine which direction the adaptive immune response will polarize. Depending on the type of pathogen and other factors, the resulting Th population may evolve into a proinflammatory (Th1), anti-inflammatory (Th2), or regulatory (Treg) phenotype.

RESOLUTION OF INFLAMMATION AND TISSUE REPAIR

The inflammatory response is often transient event. However, when the inflammation do not resolve and persist over time, then it becomes chronic. So, the resolution of inflammation is a highly well managed cellular and biochemical process to prevent progression from acute inflammation to persistent, chronic inflammation.[2,33] Several mechanisms play roles in the resolution of inflammation, including a cessation of neutrophil recruitment, a decrease in macrophage numbers and transition to the M1 phenotype, down-regulation of pro-inflammatory cytokine synthesis and growth factors expression, a decrease in the vascular smooth muscle cells (VSMC) proliferative index, and regeneration of an intact endothelium and the initiation of tissue remodeling.[33-35]

Especially, soluble factors such as pro-resolving mediators (SPMs) play a crucial role in controlling the resolution of inflammation. Key mediators include resolvins, protectins, and maresins. These mediators lead to self-limited innate responses and also promote innate microbial killing and clearance to protect tissue structure and organ function.[36] Apart from SPMs, other soluble mediators such as cytokines, cleaved extracellular domains of cytokine receptors or receptor antagonists are involved in the resolution of inflammation.[8,36] For instance, cytokines with anti-inflammatory immune function such as IL-10 and TGF-β are frequently produced during inflammation by macrophages or lymphocytes, respectively.[36] For example, cleaved extracellular domains of soluble TNFR and IL-1R restrict inflammation by neutralizing their respective cytokines. Additional anti-inflammatory factors involved in the resolution of inflammation are stress hormones such as corticosteroids, negative regulators of Toll- like-receptor signaling, and several microRNAs.[8] Additionally, regulatory T cells (Treg) produce immune suppressive cytokines such as IL-10 and TGF-β and favor resolution of inflammatory processes.[36-38]

When acute inflammatory response mechanisms fail to resolve inflammation and eliminate tissue injury, chronic inflammation occurs, and this may lead to a host of diseases, such as cardiovascular diseases, atherosclerosis, type 2 diabetes, neurodegenerative diseases, asthma, rheumatic diseases, and cancers.[2,36]

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Inflammation and Microbiota

İnflamasyon ve Mikrobiyota

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ABSTRACT The gut microbiome is a complex ecosystem in the host body. It lives in balance with its host. The peaceful coexistence of trillions of microorganisms in the human body is called symbiosis. Symbiosis is necessary for the host to live a healthy life. The intestinal barrier is a complex formation that separates the external environment from the sterile body environment. The structure and function of the intestinal barrier and the role of healthy microbiota in the protection of the intestinal barrier, as well as its effect on the local and systemic immune system, are important for understanding the relationship between inflammation and microbiota. Dysbiosis is defined as the disruption of the intestinal microbial ecosystem; the decrease in diversity of microbiota; the decline of some species and the dominance of the others. Dysbiosis leads to effects such as disruption of the energy balance in the body and triggering inflammation, which are associated with many diseases in humans. It has been shown that there are mechanistic relationships between intestinal dysbiosis and inflammation-induced obesity, atherosclerosis, autoimmune diseases and neurological disorders. The integrity of the intestinal barrier, diversities in microbial species, and the presence of metabolites such as short-chain fatty acids, trimethylamine oxide and indole derivatives of tryptophan are significant in the pathogenesis of all these diseases.

Keywords: Intestinal microbiota; immune system; short chain fatty acids; mucus layer; atherosclerosis; arthritis

ÖZET Bağırsak mikrobiyatası, konakçısı olduğu vücutla dengede olan kompleks bir ekosistemdir. İnsan vücudunda trilyonlarca mikroorganizmanın barış içinde yaşamasına simbiyozis denir. Simbiyozis konağın sağlıklı yaşaması için gereklidir. İntestinal bariyer, dış ortamla steril vücut ortamını birbirinden ayıran karmaşık bir oluşumdur. İntestinal bariyerin yapı ve fonksiyonu, sağlıklı mikrobiyatanın intestinal bariyerin korunmasındaki görevleri ve ayrıca lokal ve sistemik immün sistem üzerine etkisi mikrobiyatanın inflamasyonla ilişkisinin anlaşılmasında önemlidir. Disbiyozis, intestinal mikrobiyal ekosistemin bozulması, çeşitliliğin azalması ve bazı türlerin azalarak bazılarının dominant hale gelmesidir. Disbiyozis, vücutta enerji dengesinin bozulması ve inflamasyonun tetiklenmesi gibi etkilere yol açar; bu durum da insanlarda ortaya çıkan çok sayıda hastalık ile ilişkilendirilmiştir. İntestinal disbiyozisin inflamasyonla giden obezite, ateroskleroz, otoimmün hastalıklar ve nörolojik bozukluklar ile mekanistik ilişkilerinin mevcut olduğu gösterilmiştir. Tüm bu hastalıkların patogenezlerinde; intestinal bariyerin bütünlüğü, mikrobiyal çeşitlilikteki farklılıklar, kısa zincirli yağ asitleri, trimetilamin oksit ve triptofanın indol türevleri gibi metabolitlerin varlığı etkilidir.

Anahtar Kelimeler: İntestinal mikrobiyota; immün sistem; kısa zincirli yağ asitleri; mukus tabakası; ateroskleroz; artrit

INTESTINAL MICROBIOTA

More than 70% of all microorganisms in the human body are colonized in the intestines. The intestinal tract is a tremendous "network" which includes 10^{14} (100 000 000 000 000) microorganisms. This structure consists of bacteria, viruses, archaea, fungi, and other single-celled organisms. There are more than 50 phyla

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and more than 35000 species in the intestines of healthy individuals. Bacteriodetes (class Bacteroidia) and Firmicutes (class Clostridia) constitute 90% of the microorganisms, followed by Actinobacteria, Proteobacteria and Fusobacteria.[1] The peaceful coexistence of trillions of microorganisms in the human body is called symbiosis, and this state of balance is necessary for a healthy life. The intestinal microbiota is primarily essential for the development and maintenance of the intestines, liver and brain. Moreover, it is necessary for vitamin synthesis (vitamins B1, B2, B6, B12, biotin, nicotinic acid, folate, and vitamin K), absorption of nutrients, and regulation of the body's energy metabolism. It also performs functions such as the healthy organization of the interaction in axes such as the gut-brain axis/gut-joint axis/gut-liver axis. In addition to these main functions, intestinal microbiota has vital local effects on intestinal barrier function such as fighting against intestinal pathogens, providing nutrients to enterocytes by producing short-chain fatty acids and ensuring the survival of enterocytes. Up to 70% of the immune cells in our entire body are in our intestines. Intestinal microorganisms are necessary for the development of the host's innate and adaptive immune system. Immune system of sterile "germ-free" mice have not developed and can not elicit a host immune response until bacterial antigens are given to their blood in published studies.[2] The diversity of intestinal content and its effect on the structure and functions of the whole body makes our intestines the most active organ of our body.

INTESTINAL BARRIER AND ITS FUNCTION

Epithelial and endothelial barriers in the intestines, skin, blood vessels, respiratory tract and brain are extremely important for human health. The intestinal barrier is the vast formation between the external environment and the sterile body environment. This barrier is also necessary for healthy intestinal contents. The intestinal barrier consists of four layers: mucus layer, microorganisms and their products, epithelial cell layer and lamina propria layer. Goblet cells in the epithelial barrier constantly secrete mucus which prevents the direct contact of microorganisms in the colon with the epithelial cells. Paneth cells secrete bactericidal proteins against microorganisms. Dendritic cells identify pathogenic and commensal microorganisms in the lumen with their intestinal extensions. In this way, they send defence or tolerance signals to T regulatory (Treg) cells in the lamina propria and stimulate B cells in the lamina propria for the secretion of immunoglobulin A against pathogenic microorganisms.[3]

EFFECT OF INTESTINAL MICROBIOTA ON INTESTINAL BARRIER AND IMMUNITY

The microbiota is an essential part of the intestinal barrier. It competes with pathogens for colonization, nutrients and binding. In this way, pathogens are eliminated, and infections are prevented. It induces regulatory T-cell differentiation, thereby increasing tolerance to antigens in the lumen. They bind to Toll-like receptors (TLR), thus inducing antimicrobial protein release from Paneth cells. They also stimulate mucus secretion from Goblet cells by secreting IL-22 from lymphoid cells.

In addition to the positive effects on the intestinal barrier, microorganisms also have effects on immunity through some of the metabolites they produce. The studies show that 11-16% of metabolites in plasma are regulated by intestinal microbiota. Metabolites are produced in two ways: those produced directly by intestinal microorganisms or those excreted by the host into the intestinal lumen and processed by microorganisms to be reintroduced into the body (Table 1). These metabolites are involved in many pathways such as cell life cycle, neurobiological signaling, cholesterol and bile acid metabolism, immune response and antioxidant system responses. One of the most important of these metabolites are short-chain fatty acids (SCFA). Three SCFAs; butyrate, acetoacetate, and propionate are produced by microorganisms.[4]

The produced SCFAs, especially butyrate, have numerous regulatory effects on intestinal barrier integrity and mucosal immunity (Table 2).[5] Butyrate also induces fatty acid beta-oxidation in enterocytes by increasing PPAR-g expression in epithelial cells. In this way, ATP is produced and autophagy is inhibited in colonocytes. Meanwhile, ambient oxygen is consumed and anaerobic colonization is maintained by decreasing oxygen in the lumen.[6] Normally, obligate anaerobic bacteria are dominant in the colon. Bacteriodes, Clostridium, Bifidobacterium, and Fusobacterium genera are the most prominent obligate anaerobic bacteria.

TABLE 1: Examples of metabolites excreted by the host into the intestinal lumen and produced by processing by microorganisms in the lumen.

Short chain fatty acids (Butyrate, acetoacetate, propionate)
Secondary bile acids
Tryptophan metabolites
Polyamines
Linoleic acid metabolites
Trimethylamine-N-oxide

TABLE 2: Effects of butyrate on the mucosal barrier, enterocytes and immune system.[5]

1. Butyrate, which is produced from dietary and non-digestible fibers, provides nutrients and energy for enterocytes
2. Increasing the number of Goblet cells and mucin secretion by increasing prostaglandin production from subepithelial myofibroblasts, thereby maintaining the mucin layer
3. Tightening of tight junction structures by increasing AMPK activity
4. By binding to colonocytes, increasing anti-inflammatory IL-18 secretion from these cells and protecting colonocytes
5. Inhibition of the inflammatory cytokine NF-kappa B production through activation of PPAR γ
6. Inhibition of NF-kappa B production and suppression of proinflammatory cytokine production by inhibition of TLR4 gene expression in B cells and colonocytes
7. Increasing anti-inflammatory IL-10 secretion by binding to dendritic cells and macrophages and inhibition of pro-inflammatory cytokine production such as NO, IL-6 and IL-12 by inhibition of histone deacetylase
8. Prevention of chemotaxis of neutrophils and eosinophils to the mucosal layer and inhibition of mucosal infiltration
9. Reducing the number of activated B lymphocytes in lymph nodes in the cecum and preventing macrophage migration to these nodes
10. Stimulation of GLP-1 and PPY production from enteroendocrine L cells in the colon

Faecalibacterium prausnitzii (from the phylum Firmicutes) which is the dominant microorganism in the colon of healthy individuals, is very sensitive to oxygen and has anti-inflammatory properties. However, the coexistence of *F. prausnitzii* and *B. thetaiotaomicron* is required. Because *B. thetaiotaomicron* produces acetate from fibers and *F. prausnitzii* breaks down this acetate and converts it to butyrate. The coexistence of these two bacteria is vital for colonocytes.

Another important bacterium which has been the subject of research lately is *Akkermansia muciniphila*. *Akkermansia muciniphila* has a critical role in the regulation of the gut barrier and other homeostatic and metabolic functions. In a healthy human intestine, *Akkermansia muciniphila* makes up 3-5% of the microbial composition. In human studies, extracellular vesicles of *Akkermansia muciniphila* were found to be significantly less in stool samples of diabetic patients compared to healthy controls.[7] Furthermore, after extracellular vesicles produced by *Akkermansia muciniphila* were given to fatty diet-induced diabetic rats, the weight gain of the animals slowed down, intestinal permeability decreased, and the expression of three major tight junction proteins; occludin, zona occludens and claudin-5, increased in intestinal tissue sections.[7] In addition, *Akkermansia muciniphila* upregulates claudin 3 and occludin through the activation of TLR2 signaling and is considered a mucin-degrading species which normalizes inner mucus layer thickness.[8] According to these studies, for the optimal functioning of the intestinal barrier, not only the variety or number of microorganisms, but also the presence of specific microorganisms in the intestinal tract in a definite ratio is important.

DYSBIOSIS

Disruption of the intestinal microbial ecosystem is called dysbiosis. Dysbiosis means replacement of obligate anaerobes that are beneficial for the body with potentially more pathogenic facultative anaerobic or aerobic microorganisms and a decrease in microbial diversity. The causes of dysbiosis include the use of antibiotics, fat and carbohydrate rich diet, excess energy intake, intestinal lumen diseases, infections, systemic diseases, changes in mental status and ageing. Diversity is very important in the intestinal environment because different microorganisms in the lumen break down different dietary fibers.[1] Diversity is also essential for intestinal resistance to physiological stresses. Reduced diversity in dysbiosis causes inadequate degradation of the fibers and therefore reduced production of short-chain fatty acids. In the absence of butyrate, the previously mentioned PPAR-g stimulation does not occur and the energy yield in enterocytes shifts to anaerobic glycolysis. In this situation, the exposed oxygen is released into the lumen and produces NO by combining with L-arginine. NO provides a source of electrons for the respiration of facultative anaerobes. These facultative anaerobes (*E. coli*) begin to proliferate in an oxygen-rich environment. Tight junctions loosen and this leads to leaky gut.[9] Tight junctions which exist between enterocytes consist of four layers and allow only the passage of water and sodium. All intestinal contents, except water and sodium, must pass through enterocytes to be absorbed by the body.[10] However, loosening of the tight junction system means that the lipopolysaccharides and bacterial products in the intestinal lumen can pass into the systemic circulation (endotoxemia) and are associated with many diseases. Intestinal infec-

tions, sepsis, obesity, nutritional disorders, fatty liver disease, type 2 diabetes, inflammatory bowel diseases, autoimmune rheumatological diseases, mood disorders such as depression and premature ageing are examples of diseases with loosening in the tight junction system.[3,11]

THE RELATIONSHIP BETWEEN MICROBIOTA AND INFLAMMATION

The link between microbiota and inflammation-related diseases can not be explained as a simple cause-and-effect relationship. The underlying pathophysiology is highly complex and there is still a lot of research being done on this subject.

AUTOIMMUNE AND RHEUMATOLOGIC DISORDERS

Inflammation is prominent in autoimmune and rheumatologic disorders, and observations and research studies have been done about inflammation in these disease groups for over a century. In 1896, it was first suggested that rheumatoid arthritis (RA) develops due to mycobacterial infections. In the 1900s, the hypothesis of oral sepsis was put forward for the etiology of RA and dental procedures such as the removal of decayed teeth were performed in these patients. At the beginning of the 2000s, the relationship between periodontal disease and RA has been demonstrated. Treatment of periodontitis has been shown to reduce rheumatological complaints in these patients. In the literature, it has been shown that the diversity of microbiota decreases and certain pathogenic organisms predominate in rheumatological diseases such as rheumatoid arthritis and systemic lupus erythematosus.[12,13]

As in many autoimmune and rheumatologic disorders, molecular mimicry was thought to be responsible for the pathogenesis of these diseases. According to this theory, autoimmunity is triggered in the body due to the molecular similarity between bacterial antigens and synovial tissue proteins.[11,14] Another theory in the pathogenesis is the exogenous enzymes of the dysbiotic population causing post-translational modification of host synovial proteins.[15] In particular, the conversion of arginine residue of proteins into citrulline, which is called citrullination. *Porphyromonas gingivalis* is the only prokaryote that contains the endogenous peptidylarginine deiminase enzyme, which converts the arginine residue to citrulline. Therefore, it is considered a major factor in the development of RA.[16] Citrullinated proteins gain neo-antigen properties, reduce immune tolerance and trigger autoimmunity by resembling bacterial proteins. The presence of anti-citrullinated protein antibodies has also been demonstrated in RA patients.

ATHEROSCLEROSIS

Another disease group associated with chronic inflammation is atherosclerosis.[17] When the microbiota of individuals with and without atherosclerosis was compared, differences were found between their microbiota.[18] Dysbiosis in patients with atherosclerosis activates the NF-kappa B signaling pathway as a result of leaky gut and endotoxemia. In this way, pro-inflammatory cytokine production, as well as TH17 production and IL-17 synthesis increase due to damage to the mucosal barrier, in addition to increase in trimethylamine synthesis. As a result, atherosclerosis occurs due to combination multiple pathologies such as the development of endothelial dysfunction and vasoconstriction, decreased cholesterol elimination, uptake of lipids by macrophages and foam cell formation, increased platelet hyperactivity and aggregation, and development of thrombosis.

Trimethylamine (TMA) production is associated with atherosclerosis by different mechanisms. TMA is produced by bacteria such as *Escherichia fergusonii, Proteus mirabilis*, and *Proteus penneri* from choline, phosphatidylcholine, and L-carnitine which are obtained in dietary intake of meat, eggs, seafood, and fatty foods. Produced TMA is converted to trimethylamine oxide (TMAO) by flavin monooxygenase 3 (FMO3) enzyme in hepatocytes and released into the systemic circulation.[19] A correlation was found between the increase in TMAO in the body and the development of atherosclerosis. TMAO alters cholesterol and bile acid metabolism, triggers inflammation by increasing TNF-alpha and IL-6 production, increases ROS production, decreases endothelial-protecting anti-inflammatory IL-10 production, activates platelets and macrophages, induces foam cell formation by CD36 and SR-A1 receptor “upregulation”.[19,20] In addition to all these, it impairs the self-renewal capacity of the damaged endothelium.[18] In addition, TMAO level was found to be associated with insulin resistance and hypertension.[5,17]

It is reported that butyrate has a preventive effect on the development of atherosclerosis.[19] In the literature, it has been determined that bacteria such as Roseburia and Eubacterium, which produce less butyrate than the others, are dominant in the intestines of individuals with atherosclerosis.[21,22] In addition, butyrate administration to mice by the intestinal route has been shown to inhibit inflammation and the development of atherosclerosis.[23] It is believed that this effect of butyrate occurs by inhibiting the production of vascular cell adhesion molecules and NF-kappa B activity.[24]

OBESITY AND FATTY LIVER DISEASE

Obesity is one of the diseases associated with both inflammation and dysbiosis. Over-nutrition with fat and energy content causes dysbiosis. On the other hand, dysbiosis causes extra energy to be taken into the body by releasing approximately 150 Kcal of energy per day from undigested foods and causes more weight gain by affecting the synthesis and activities of proteins related to energy metabolism and inflammation.[25]

Endotoxins (lipopolysaccharides, LPS) of gram-negative bacteria cause secretion of cytokines and chemokines (TNF-α, TGF-β, IL-6, IL-10), and inflammation in the intestinal system and adipose tissue by stimulating hepatic toll-like receptors (TLR4 and TLR9).[25,26] Triggered inflammation is associated with the occurrence of many obesity-related complications, especially fatty liver disease and non-alcoholic steatohepatitis.

Bacteria in the healthy microbiota produce indole derivatives (indole-3-aldehyde, indole-3- acetic acid, and indole-3-lactic acid) from dietary tryptophan. These products interact with the aryl hydrocarbon receptor (AHR), reducing intestinal permeability and strengthening the barrier.[26,27] AHR receptors are crucial transcription factors expressed in many immune cells and their stimulation is essential for innate and adaptive immunity. Lactobacilli and Bifidobacteria are the only bacteria that can produce indole from tryptophan in the intestinal tract. In relation to dysbiosis, the decrease in number of these indole-producing bacteria results in increased intestinal permeability and inflammation.[26]

NEUROINFLAMMATION

Central nervous system (CNS) neuroinflammation resulting from injury, infection, or disruption of neural tissue integrity is usually self-limited, and mediates tissue defense and repair without harming the host. However, in some situations inflammatory responses can become chronic or detrimental. In these cases, neuroinflammatory diseases occur.

Intestinal microorganisms affect both the enteric nervous system and the central nervous system through signaling.[28] Some CNS diseases such as stroke, neurodegeneration, and depression have been shown to be associated with intestinal dysbiosis and inflammation. Possible mechanisms include inflammatory cytokines, toxic metabolites, or induced peripheral immune cells emerging in the body as a result of dysbiosis, reaching the brain through the circulation or transmitting signals via the *N. vagus*.

Bacterial lipopolysaccharides are potent stimulators that create local and systemic immune responses. It has been shown that there is an increment in LPS-producing bacteria genes in Parkinson's patients and TLR4 expression is increased in colon biopsies of patients.[29] TLR4 knockout mice have been shown to have less intestinal inflammation, reduced microglial activation, reduced degeneration in the substantia nigra, and less motor dysfunction when mice are given the neurotoxin rotenone.[29]

In a previous study, CNS inflammation and encephalomyelitis scores were decreased when indole metabolites of tryptophan (indole, indoxyl-3-sulfate, indole-3-propionic acid and indole-3-aldehyde) were given to animals with autoimmune encephalitis model and subsequent ampicillin induced dysbiosis.[30] It has been noticed that this effect occurs through increasing the expression of AHR in astrocytes.[30] Similarly, in many animal studies using SCFA, the amount of SCFA in the body, and neuroinflammation & pro-inflammatory cytokine levels were found to be inversely proportional.[28] In the meta-analysis of studies examining the microbiota of patients with Parkinson's disease, it was found that the levels of the Lachnospiraceae family and the genus Faecalibacterium, both of which produce SCFA, were significantly lower than in healthy individuals.[31] It has been suggested that the decrease in SCFA levels in these patients may be associated with the pathogenesis of the disease.[31]

The microbiome can also generate molecules that mimic host proteins. Capsular carbohydrates and peptides of microorganisms were found to be similar to the structure of the myelin oligodendrocyte glycoprotein (MOG)-specific T cells.[32,33] In patients with autoimmune encephalomyelitis, it has been determined that *Lactobacillus reuteri* and *Allobaculum spp.* increase IL-17A-producing CD4+ T cells in the intestines and exacerbate the findings of encephalomyelitis.[33] It is thought that intestinal T cells migrating to the CNS can acquire auto-reactivtiy and contribute to the emergence of neuroinflammation-related CNS disease by the interaction of bacterial products.[28]

As mentioned above, TMAO formation due to dysbiosis also contributes to the development of stroke by creating a predisposition to atherosclerosis.[34]

THE EFFECT OF USING TREATMENTS REGULATING THE MICROBIOTA ON INFLAMMATION AND RELATED DISEASES

Hypothetically, correcting the impaired intestinal microbiota can repair the damaged mechanisms by correcting inflammation and can prevent chronic diseases associated

with inflammation.[35] There are many studies on this subject. According to the results of randomized controlled studies and their meta-analysis, when probiotics are used, acute phase reactants (CRP, hsCRP) generally decrease, TNF-alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-12, MDA levels decrease; thrombomodulin, VCAM, endothelial growth factor levels decrease, whereas anti-inflammatory IL-10 level, glutathione level and total antioxidant capacity increase. These effects were found to be more prominent, especially in patient groups with obesity, diabetes, hypertension and fatty liver disease. In the healthy group, these parameters generally do not change with probiotic supplementation.[17,35]

As in most of the probiotic studies in the literature, inflammation-related probiotic studies have varying results. While some meta-analyses revealed the improvement of some parameters with probiotic supplementation in inflammation-related diseases like arthritis, fatty liver disease, cirrhosis, and inflammatory bowel disease groups, no changes were observed in other disease groups.[36] In some studies emphasizing the dosing of the probiotics, it has been shown that the positive effects can be seen when the preparations containing a large number of bacteria are used at high doses ($1x10^{10}$ cfu/day), but no effect is found at low doses ($2.5x10^{9}$ cfu/day). In some studies, some bacterial species, such as lactobacilli (*L. acidophilus, L. casei*) and bifidobacteria (*B. lactis, B. bifidum*) had a significant effect, while others did not. In some meta-analyses, the age and duration of using probiotics (for example, in patients aged >50 years, a decrease in malondialdehyde levels after >8 weeks of use) were emphasized.[37] Although there are different responses in all these laboratory parameters, the effects of probiotic use on some clinical indicators such as blood pressure, TMAO levels, cardiac or atherosclerotic parameters have not been demonstrated. Most probiotic studies have significant methodological differences. The use of different probiotics, their use alone or in combinations, the application of different doses and durations, the inhomogeneity of the patient groups, and the presence of concomitant diseases (such as obesity, diabetes, fatty liver disease) significantly affect the results of the studies.[20,35] Given all the different study results, there is currently no clear data to support the routine use of probiotics in inflammatory neurodegenerative diseases, atherosclerosis, obesity, type 2 diabetes or rheumatic diseases.

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Effects of Inflammation on Drug Metabolism and Pharmacogenetic Factors

İnflamasyonun İlaç Metabolizması Üzerine Etkileri ve Farmakogenetik Faktörler

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ABSTRACT Variability in drug metabolism can alter drug response and toxicity in human. Cytochrome P450 (CYP) enzymes play the most prominent role in drug metabolism and their activities are regulated by various mechanisms. The activities of drug metabolizing enzymes can be altered by genetic or non-genetic factors. Inflammation and chronic inflammatory diseases have been identified as important non-genetic factors regulating drug-metabolizing enzymes. Such effects have been associated with inhibition of CYP activity and expression due to the systemic cytokines. Increase in inflammatory markers such as CRP, IL-6 and TNF-alpha in COVID-19 infection has also been associated with decrease in activities of some CYP enzymes. Both inflammation and genetic variability may interact to alter drug response and may cause a mismatch between the predicted drug response and the actual phenotype. The phenomenon is called phenoconversion.

Keywords: Cytochrome P-450 enzyme system; genetic polymorphism; inflammation; metabolism; pharmacogenetics

ÖZET İlaç yanıtında ve yan etkilerinde gözlenen değişkenlik ilaç metabolizmasındaki farklılıklardan kaynaklanabilir. İlaçların karaciğerdeki ve karaciğer dışındaki metabolizmasından sorumlu en önemli enzim grubu sitokrom P450 monooksijenaz (CYP) enzimleridir. Bu enzimlerin aktiviteleri genetik veya genetik olmayan faktörler tarafından değiştirilebilir. İnflamasyon ve kronik inflamatuar hastalıklar ilaç metabolizmasından sorumlu enzimlerin aktivitelerini değiştiren faktörler arasındadır. İnflamasyon varlığında gözlenen CYP enzim inhibisyonları, sistemik sitokin düzeylerinin artışıyla ilişkilendirilmiştir. COVİD-19 enfeksiyonu geçiren hastalarda da CRP, İL-6 ve TNF-alfa düzeylerinde görülen artış, bazı CYP enzim aktivitelerinde azalma ile ilişkili bulunmuştur. İnflamasyon ve kronik inflamatuar hastalıklar kişilerin ilaca verdiği yanıtları değiştirebilir. Böylece bireyin genotipine göre öngörülen ilaç yanıtı ile ilaca verilen yanıt birbirini tutmayabilir. Bu kavram, fenokonversiyon (fenotip kayması) olarak adlandırılır.

Anahtar Kelimeler: Sitokrom P-450 enzim sistemi; genetik polimorfizm; inflamasyon; metabolizma; farmakogenetik

OVERVIEW OF DRUG METABOLISM AND CYTOCHROME P-450 (CYP) ENZYME SYSTEM

The aim of drug metabolism, or biotransformation, is to change the drug molecules into more ionized and hydrophilic inactive metabolites for an easier excretion from the body by using some enzymatic reactions. While the liver is the major organ for biotransformation, some extrahepatic mechanisms are also responsible for this process.[1] The enzymatic breakdown of drug molecules is usually categorized into *phase I* and *phase II* reactions. Hydrolytic and oxidation/reduction reactions are usually classified as the *phase I* reactions while conjugative reactions are commonly de-

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scribed as in the *phase II* reactions. Distinct enzymes have been described for diverse reactions in these two phases. The *phase I* enzymes mainly include cytochrome P450 (CYP) system, which allows to perform many oxidative reactions. Alcohol dehydrogenase (ADH) and epoxide hydrolase (EH) can be given as examples for non-CYP enzymes included in *phase I* metabolism. In human, the majority of drug metabolizing enzymes belong to CYP1, CYP2 and CYP3 families and CYP3A4/5 plays a major role for biotransformation of many drugs (Figure 1).[2]

In the nomenclature of CYP enzymes, the first number defines the family class of the enzyme, and the first letter coming afterwards defines the subgroup name. The second number following the subgroup letter indicates the specific isoform name of an individual enzyme, such as CYP1A2, CYP2D6 or CYP3A4.[3]

EFFECTS OF INFLAMMATION ON DRUG METABOLISM

Variability in drug metabolism and elimination can alter drug response and toxicity in human. The sources of this variability may be both genetic factors and non-genetic factors, such as diseases, nutrition status and etc.[5] If the metabolism for a drug slows down in a human subject due to a genetic or non-genetic factor, the drug would accumulate more than the expected in the patient's plasma, and the effect of drug may increase. Also, the duration of the drug effect may prolong and the toxic effects may appear. On the contrary, if the metabolism rate for a drug accelerates the drug molecule would be eliminated more rapidly, and the therapeutic effects and the duration of the drug effect may diminish.[6]

It has been shown in many studies that acute or chronic inflammation can inhibit the level and the activity of major *phase I* enzymes including CYP enzymes, and also few some *phase II* enzymes and drug transporters, such as multidrug resistance protein-1 (MDR1). This inhibition may result from alteration of the expression of the genes responsible for encoding of the proteins involved, or from some epigenetic changes associated with reduced activities of the enzymes or the transporters.[7]

It has been reported that DNA methylation levels of the genes coding for metabolizer enzymes such as CYP1A2, CYP2C19, CYP2D6, GSTT1 (Glutathione S-transferase theta-1) and SULT1A1 (Sulfotransferase 1A1) correlate with reduced expression levels of these genes in human.[8] CYP inhibition may result from various biochemical pathways involving inflammatory cytokines like IL-1β, IL-2, IL-6, IFN-γ etc. For example, it was reported

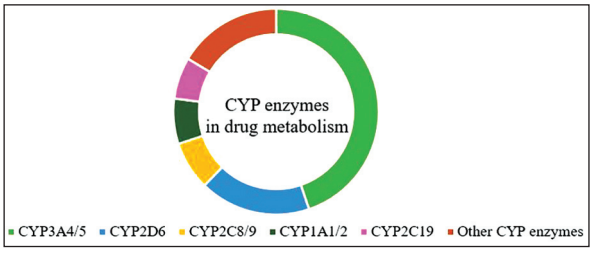


FIGURE 1: Contribution of CYP enzymes in metabolism of drugs used in clinical practice. The relative size of each portion represents an average percentage of drugs metabolized by CYP enzymes. Most drugs are metabolized by CYP3A4/5. CYP2D6, 2C8/9, 1A1/2 and 2C19 also play important roles for many drugs (taken from reference with modifications).[4]

that IL-6 caused downregulation of CYP enzymes through various biochemical pathways such as the Janus kinase (JAK)/STAT protein-3 (STAT3) pathway, the mitogen activated protein kinase (MAPK), ERK1/2 pathway and the PI3K/AKT pathway.[9] The inhibitory effect of IL-1β on CYP enzymes was associated with NF-κB pathway.[10] IL-1β was demonstrated to increase the levels of IL-6, which can suppress the expression of CYP enzymes.[11] Inhibition of CYP enzymes due to activated inflammatory pathways was associated with downregulation of transcriptional factors like pregnane X (PXR) nuclear receptors, or activities of hepatocyte nuclear factors HNF-1α and HNF-4α, and upregulation of transcriptional factors like C/EBPβ in liver.[12-14] Post-transcriptional mechanisms were also associated with CYP inhibition in the liver via pathways involved in regulation of micro-RNAs (miRNA), such as miR-224, miR-155 and miR-452.[15]

in vitro EXPERIMENTAL STUDIES ABOUT THE EFFECTS OF INFLAMMATION ON DRUG METABOLISM

The effects of inflammation have been studied in many *in vitro* models and human tissue samples. For example, in human hepatocyte cell lines, IL-6 mediated suppression of CYP3A4 mRNA level and the enzymatic activity of CYP3A4 was demonstrated by using conversion of testosterone to 6-β-OH-testosterone in a concentration-dependent way (Table 1).[16]

Likewise, IL-1β, IFN-γ, IL-6 and TNF-α suppress mRNA levels and activity of many CYP enzymes *in vitro* in human hepatocyte cell lines (Table 2, 3). From 1,5-folds to above 5-folds inhibitory effects compared to normal status were observed in many *in vitro* studies. IL-1β and IL-6 inhibit some CYP enzymes in a concentration-dependent manner to 100-folds inhibition of mRNA levels.

TABLE 1: CYP3A4 mRNA levels and activity due to IL-6 exposure in human hepatocytes.[16]

Inhibition of CYP3A4 mRNA and activity	
IL-6 concentration (ng/ml)	**CYP3A4 residual activity (Approximate % of control)**
10^{-2}	90
10^{-1}	80
1	60
10	30

TABLE 2: The effect 10 ng/mL concentration of cytokines on CYP3A4, CYP2D6 and CYP2C9 mRNAs and enzymatic activities in human hepatocytes *(taken from reference 5 with modifications)*.

	Suppression level					
Cytokine	**CYP3A4**		**CYP2D6**		**CYP2C9**	
IL-1β	*mRNA*	*Activity*	*mRNA*	*Activity*	*mRNA*	*Activity*
	>5x	> 5x	2x-5x	-	2x-5x	-
IL-6	*mRNA*	*Activity*	*mRNA*	*Activity*	*mRNA*	*Activity*
	>5x	> 5x	1,5x-2x	1,5x-2x	2x-5x	1,5x-2x
IFN-γ	*mRNA*	*Activity*	*mRNA*	*Activity*	*mRNA*	*Activity*
	2x-5x	-	-	-	1,5x-2x	-
TNF-α	*mRNA*	*Activity*	*mRNA*	*Activity*	*mRNA*	*Activity*
	> 5x	2x-5x	1,5x-2x	1,5x-2x	-	-

1,5x-2x: 1,5-2-fold; 2x-5x: 2-5-fold; > 5x: > 5-fold suppression.

TABLE 3: The effect of cytokines (10 ng/mL each) on CYP2C19 and CYP1A2 mRNAs and enzymatic activities in human hepatocytes suppression *(taken from reference 5 with modifications)*.

	Suppression level			
Cytokine	**CYP2C19**		**CYP1A2**	
IL-1β	*mRNA*	*Activity*	*mRNA*	*Activity*
	1,5x-2x	-	2x-5x	2x-5x
IL-6	*mRNA*	*Activity*	*mRNA*	*Activity*
	2x-5x	2x-5x	2x-5x	2x-5x
IFN-γ	*mRNA*	*Activity*	*mRNA*	*Activity*
	-	-	-	-
TNF-α	*mRNA*	*Activity*	*mRNA*	*Activity*
	-	>5x	1,5x-2x	2x-5x

1,5x-2x: 1,5-2-fold; 2x-5x: 2-5-fold; > 5x: > 5-fold suppression.

Along with drug metabolizing enzymes; mRNAs and protein levels of many drug transporters in human hepatocyte cell lines have been suppressed by IL-1β, IFN-γ, IL-6 and TNF-α, *in vitro*. Inhibition was evident at a range of 1,5- to more than 5-folds depending on the type and concentration of cytokines (Table 4).

in vivo EXPERIMENTAL STUDIES ABOUT THE EFFECTS OF INFLAMMATION ON DRUG METABOLISM

Inhibitory effects of cytokines released during acute and chronic inflammatory processes have been investigated in many animal models. Subjects could be wild-type or

TABLE 4: Effects of pro-inflammatory cytokines in the concentration range of 1-100 ng/ml on drug transporter protein levels and their mRNA expressions in human hepatocyte cell lines *(taken from reference 5 with modifications)*.

	Suppression level							
Cytokine	**MDR1**		**BCRP**		**MRP2**		**OATP1B1**	
IL-1β	*mRNA*		*mRNA*		*mRNA*		*mRNA*	
	-		2x-5x		1,5x-2x		> 5x	
IL-6	*mRNA*	*Protein*	*mRNA*	*Protein*	*mRNA*	*Protein*	*mRNA*	*Protein*
	1,5x-2x	-	1,5x-2x	1,5x-2x	1,5x-2x	1,5x-2x	> 5x	1,5x-2x
IFN-γ	*mRNA*		*mRNA*		*mRNA*		*mRNA*	
	1,5x-2x		1,5x-2x		1,5x-2x		2x-5x	
TNF-α	*mRNA*	*Protein*	*mRNA*	*Protein*	*mRNA*	*Protein*	*mRNA*	*Protein*
	1,5x-2x	-	-	-	-	-	2x-5x	1,5x-2x

The symbol (x) indicates folds of inhibition comparing to state of cytokine pre-exposure. BCRP: Breast cancer resistance protein. MRP2: Multidrug resistance-associated protein 2. OATP1B1: Organic anion transporter protein 1B1.

knockout animals for several genes expressing proteins in inflammatory pathways.[17,18] Many CYP enzyme subtypes have been found to be suppressed by cytokines in these models, however some CYP enzymes remain unchanged (Table 5).

In addition to *in vivo* experimental animal studies, CYP inhibition via inflammatory cytokines were also observed in human studies. In human subjects, when high doses of human recombinant IL-2 were administered systemically, the hepatic content of CYP enzymes in non-tumorous liver tissues from cancer patients were decreased as compared to healthy control subjects (Table 6).[20]

Pharmacokinetic parameters measured by using specific probe (substrate) drugs for several CYP enzyme activities have shown that some of activities were decreased in subjects with chronic or acute inflammatory conditions, as summarized in Tables 7 and 8. For example, in Behçet's disease our previous studies have shown that the activities of CYP2C9 and CYP2C19 were decreased.[21,22] While inhibitory effects were generally observed in many of these type of studies, increase (Table 8) or no effects in activity levels were also reported.[4]

TABLE 5: Effects of pro-inflammatory cytokines on CYP enzymes in several experimental animal models of acute and chronic inflammation *(taken from reference 19 with modifications)*.

Inflammatory stimulus	**Animal species**	**Released cytokines**	**Liver CYP**	**1A**	**3A**	**2c11[a]**	**2c22[b]**
Lipopolysaccharide (LPS)	129s1/svlmJ mice	IL-1β, IL-6 and TNF-α	mRNA	↓	↓	↓	-
			Protein	-	↓	-	-
			Activity	-	-	-	-
High-dose (>5 mg/kg) LPS	DBA-2 mice	IL-1β, IL-6, TNF-α, IFN-γ, IL-2, IL-4, IL-10 and IL-17	mRNA	-	-	-	-
			Protein	-	-	-	-
			Activity	↓	-	-	-
Citrobacter rodentium	C3H/HeOuJ mice	IL-1β, IL-6 and TNF-α	mRNA	-	↓	↓	-
			Protein	-	-	↓	-
			Activity	-	↓	-	-
Collagen antibody-induced arthritis	BALB/c mice	IL-1β, IL-6, IL-2 and SAA	mRNA	↓	↓	↓	-
			Protein	-	-	-	-
			Activity	↓	↓	↓	-
Multiple challenges with ovalbumin	ICR mice	-	mRNA	-	-	-	-
			Protein	-	-	-	-
			Activity	↓	↓	↓	-

SAA: Serum amyloid A protein. [a]Human CYP2C9 corresponds to murine Cyp2c11. [b]Human CYP2C19 corresponds to murine Cyp2c22. ↓ indicates suppression.

TABLE 6: CYP isozymes in microsomal samples from non-tumorous liver tissues when different doses of IL-2 were administered as compared to the controls *(taken from reference 20 with modifications)*.

		CYP isozymes (% of control, means±standard errors of the mean)			
IL-2 units (10^6/m^2/day)	**Number of subjects**	**CYP3A**	**CYP2C**	**CYP1A2**	**CYP2E1**
0	7	100±43	100±43	100±37	100±27
3 or 6	5	69±21	91±30	101±42	59±26
9 or 12	6	39±6	45±22	37±17	60±21

TABLE 7: Impact on CYP2C9 enzyme activities of chronic inflammatory diseases and acute inflammatory conditions *(taken from reference 5 with modifications)*.

Disease	**Inhibitory effect on CYP2C9 enzyme activity with the use of probe drug**		
Chronic inflammatory diseases	***Activity***	***Probe drug***	***Pharmacokinetic parameters***
Behçet's disease	1,5x-2x	Losartan	Drug/metabolite ratio
Cancer	-	Tolbutamide	AUC, CL, Cmax
Type II Diabetes mellitus	-		Drug/metabolite ratio
Rheumatoid arthritis	-	Losartan	AUC
Acute inflammatory diseases	***Activity***	***Probe drug***	***Pharmacokinetic parameters***
Parasitic diseases	-	Losartan	Drug/metabolite ratio
Severe injuries	1,5x-2x	Flurbiprofen	Urinary drug recovery

The symbol (x) is the fold number of inhibitory effect compared to the activity in normal situation. C_{max}: Maximum concentration. AUC: Area under the curve of the concentration-time graph. CL: Clearance.

TABLE 8: Impact on CYP2C19 enzyme activities of chronic inflammatory diseases and acute inflammatory conditions *(taken from reference 5 with modifications)*.

Disease	**Inhibitory effect on CYP2C19 enzyme activity with the use of probe drug**		
Chronic inflammatory diseases	***Activity***	***Probe drug***	***Pharmacokinetic parameters***
Behçet's disease	↓ 2x-5x	Lansoprazole	Drug/metabolite ratio
Cancer	-	Omeprazole	
Type II Diabetes mellitus	↓ 1,5x-2x		
Acute inflammatory diseases	**Activity**	**Probe drug**	**Pharmacokinetic parameters**
Parasitic diseases	↑ 1,5x-2x	Omeprazole	Drug/metabolite ratio
Severe injuries	↓ 2x-5x	Mephenytoin	Urinary drug recovery

↓ indicates decrease in activity. ↑ indicates increase in activity. The symbol (x) is the fold number of inhibitory or increasing effect compared to the activity in normal situation.

Interestingly, when the inflammatory process was inhibited *in vivo* by an antagonist in patients with chronic inflammatory disorders, CYP enzyme activities were restored. For example, when rheumatoid arthritis patients were systemically administered IL-6 antagonists, CYP3A4 activity levels in the patients were elevated by an average of 2-3 folds, as compared to the levels before the antagonist treatment (Table 9).[5]

PHARMACOGENETIC FACTORS ASSOCIATED WITH EFFECTS OF INFLAMMATION ON DRUG METABOLISM

Pharmacogenetics is the field of science that studies how genetic variations may affect the drug response among individuals. Many of CYP enzymes show genetic polymorphism. Among those enzymes, CYP 2D6, 2C9 and 2C19

TABLE 9: The reversibility of the inhibitory effect on CYP3A4 enzyme activity in inflammatory diseases *(taken from reference5 with modifications)*.

Effect on CYP3A4 enzyme activity with the use of probe drug				
Antagonist	Target	Inflammatory disease	Activity	Probe drug
Sirukumab	IL-6	Rheumatoid arthritis	↑ 1,25x-2x	Midazolam
Tocilizumab	IL-6R		↑ 1,25x-5x	Simvastatin

↑ indicates increase in activity. The symbol (x) is the fold number of increasing effect compared to the suppressed activity of CYP3A4 in inflammatory diseases. R: Receptor.

are polymorphic enzymes. As for an example, CYP2C19 is a highly polymorphic gene. Metabolic phenotypes have been defined for several drugs, eg. for voriconazole, in order to achieve pharmacotherapy goals in clinics (Table 10).[23]

In a study with 34 patients treated for an invasive fungal infection by voriconazole, it was established that the effect of inflammation was changed with the CYP2C19 genotype.[24] Also, metabolic activity of CYP2C9 was evaluated from blood samples of patients with hepatitis C virus (HCV) infection and healthy individuals. The shift in metabolic ratio due to inflammation between healthy volunteers and HCV-positive patients was the largest for normal metabolizers (21.1-fold change), followed by *intermediate metabolizers* (12.4-fold change).[19]

Despite accumulation of knowledge on how genetic polymorphisms of CYP enzymes affect drug metabolism, it's still unclear how these genetic polymorphisms may modulate the impact of inflammation on drug metabolism, and this issue need to be clarified by more studies in human.

THE CONCEPT OF PHENOCONVERSION:

Phenoconversion is a condition when both inflammation and genetic variability interact to alter drug response and cause a mismatch between the predicted drug response phenotype and the actual phenotype (Figure 2).

The reasons that may induce phenoconversion may be co-administration of a medication which can inhibit or induce CYP enzymes and co-morbidities such as inflammatory diseases.[26] For instance, when a patient with CYP2C9 genotype-based normal metabolizer phenotype is treated with a medication that inhibits the enzyme (e.g. fluvoxamine) or has a new-onset inflammatory disease, the metabolic phenotype of the patient may change to poor metabolizer status, i.e. a shift of the phenotype. Some of inhibitors of several CYP enzymes are shown in Table 11.

Phenoconversion due to inhibition of DMETs is a transient effect. The duration of the conversion may depend on the type of inhibitor (examples in Table 11) or the susceptibility of the patient.[25]

TABLE 10: Genotype-based likely phenotypes of CYP2C19 for voriconazole therapy *(taken from Clinical Pharmacogenetics and Implementation Consortium (CPIC) and reference23 with modifications)*.

Diplotype examples	Likely phenotype	Comments
*CYP2C19 *17/*17*	CYP2C19 ultrarapid metabolizer (UM)	Recommendation for an alternative antifungal agent substitute to voriconazole because of inadequate therapeutic levels
*CYP2C19 *1/*17*	CYP2C19 rapid metabolizer (RM)	Recommendation for an alternative antifungal agent substitute to voriconazole because of inadequate therapeutic levels
*CYP2C19 *1/*1*	CYP2C19 normal metabolizer (NM)	Standard dosing of voriconazole
*CYP2C19 *1/*2* *CYP2C19 *1/*3* *CYP2C19 *2/*17*	CYP2C19 intermediate metabolizer (IM)	Standard dosing of voriconazole
*CYP2C19 *2/*2* *CYP2C19 *2/*3* *CYP2C19 *3/*3*	CYP2C19 poor metabolizer (PM)	Recommendation for an alternative antifungal agent substitute to voriconazole in order to avoid drug toxicity due to poor metabolism

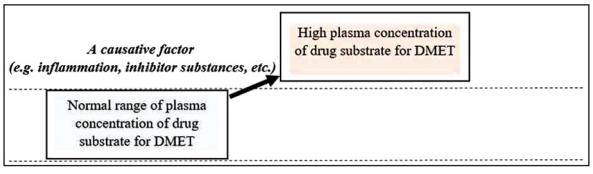


FIGURE 2: A representation of phenoconversion via a factor like inflammation.[25]
DMET: Drug metabolizing enzymes and drug transporter proteins.

TABLE 11: Examples for inhibitors for some CYP enzymes *(taken from reference 25 with modifications)*.

CYP	Inhibitors
2D6	Diphenhydramine
	Fluoxetine
	Ritonavir
	Terbinafine
2C9	Amiodarone
	Diclofenac
	Fluvoxamine
	Isoniazid
2C19	Ketoconazole
	Topiramate
3A4/5	Erythromycin
	Fluconazole

COVID-19, INFLAMMATION AND THEIR EFFECTS ON DRUG METABOLISM

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) was firstly reported in December 2019 and the disease caused by this virus was named as coronavirus disease 2019 (COVID-19). The manifestations of disease symptoms range from asymptomatic to severe disease or death.[27,28] Angiotensin-converting enzyme receptor 2 (ACE-2), is the target of SARS-Cov-2 to enter the cell. ACE-2 receptor is expressed in lungs, heart, liver, kidney, intestine and testes.[29] After endocytosis of virus, the immune system activates to eradicate it. In more than 85% of patients, this inflammation plays a role in removal of virus and healing processes. But in 10-15% of COVID-19 patients, acute respiratory distress syndrome (ARDS) or severe disease may arise because of hyper-inflammation.[30]

Inflammatory processes may be divided to three phases during SARS-Cov-2 infection:

Phase I. Invasion phase of virus: Spike protein (S-protein) of the virus binds with ACE2 receptor with the help of transmembrane protease serine 2 (TMPRSS2). After endocytosis, virus replicates itself and by cytopathic effect induce injury and pyroptosis. So, pathogens associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) release. Pattern-recognition receptors (PRRs) of the adjacent epithelial cells and alveolar macrophages recognize DAMPs and PAMPs and then activate the innate immunity.[31,32]

Phase II. Inflammation phase: PRRs trigger NF-kB and type I interferon (IFN-I) release. Also after recognition of DAMPs (heat shock protein, ATP or high mobility group box 1 protein (HMGB1)), adjacent epithelial cells, endothelial cells and macrophages induce proinflammatory mediators, such as TNF-a, IFN-γ, IL-1β, IL-2, IL-6, and the overexpression of adhesion molecules (ICAM-1, VCAM-1). SARS-Cov-2 activates lectin and classical pathway of complement system. All these processes cause pulmonary recruitment of lymphocytes.[33] Cytotoxic T cells are activated by antigen-presenting cells and destroy infected cells. B cells also generate neutralizing antibodies especially targeting S protein.[33] After the clearance of virus immune-responses gradually resolve. During the inflammatory phase, regulator cells like T_{reg}, produce anti-inflammatory cytokines like IL-10 and TGF-B to antagonize hyper-inflammation.[34]

Phase III. Hyper-inflammation phase (Cytokine storm): In some patients, the regulation of immune system fails and hyper-inflammatory stage of COVID -19, called cytokine storm, occurs.[35] During this phase procalcitonin, CRP, D-dimer, ferritin, TNF-α, IFN-γ, G-CSF, GM-CSF, IL-2, IL-6, IL-10, and MCP-1 have very high levels in the serum of patients.[34] In these patients the balance between proinflammatory and anti-inflammatory cytokines are broken. For example, anti-inflammatory mediators like IL-10, may be produced but are not sufficient to antagonize the hyper-inflammation.[34] NF-κB may induce the production of TNF-α, IFN-γ, IL-2 and IL-6 and this may upregulate NF-κB signaling. In severe COVID-19 patients, the level of IFN-I was found low. Viral proteins such as M, N, open reading frame 3a (ORF3a) and ORF6 proteins may probably inhibit the IFN-I signaling. Also, M1 macrophages and CD4 T cells may overproduce cytokines that can exacerbate the inflammation.[31,34,36]

As a result of hyper-inflammation, systemic microvascular injury and immunothrombosis with activation of the hematolymphoid system were shown in autopsy series of deceased COVID-19 patients.[37]

Many anti-inflammatory drugs were investigated in COVID-19 because of the importance of inflammation in COVID-19. These include IL-6 inhibitor (tocilizumab), IL-1β antagonist (anakinra), IL-12/23 inhibitor (ustekinumab),

IFN-α inhibitor (sifalimumab), TNF-α inhibitor (adalimumab), IL-17A inhibitor (brodalumab), Janus kinase (JAK) inhibitor (baricitinib) and immunomodulators (chloroquine, hydroxychloroquine, corticosteroids and vitamin therapy).[38] National Institute of Health (NIH) guideline for COVID -19 recommends the use of a combination of 2 immunomodulators (either dexamethasone plus baricitinib [AI] or dexamethasone plus tocilizumab [BIIa]) for patients who need oxygen supplementation.[39]

In a recent study in COVID-19 patients, the activities of CYP1A2, CYP2C19, and CYP3A enzymes were measured by using enzyme-selective probe (substrate) drugs which were metabolized by specific enzymes, *in vivo*. It was reported that the levels of inflammatory markers such as CRP, IL-6 and TNF-alpha were elevated in COVID-19 patients and the activities of CYP1A2, CYP2C19, and CYP3A enzymes were decreased by 52.6%, 74.7%, and 22.8%, respectively, in the active infection period of the disease.[40]

In conclusion, both inflammation and pharmacogenetic variations may affect the outcomes of drug therapy. It would be important to clarify the mechanisms of inflammatory process on drug metabolism in order to achieve an adequate target in drug therapy of patients in the context of personalized medicine.

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Metabolic Syndrome, Obesity and Inflammation

Metabolik Sendrom, Obezite ve İnflamasyon

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ABSTRACT Obesity is recognized as a serious public health problem, as it increases the risks of multiple diseases, such as diabetes, heart disease, high blood pressure, and some cancers. Metabolic syndrome consists of several metabolic abnormalities, including intraabdominal obesity, hyperglycemia, and hypertension. Inflammation is a natural part of the immune system and is the body's natural defensive response to many harmful, foreign and destructive effects. However, when inflammation develops against its tissues, chronic inflammation develops. Obesity causes low-grade chronic inflammation. Adipose tissue is one of the most important endocrine organs and it releases many inflammatory mediators such as Interleukin-1, Interleukin-6, Tumor Necrosis Factor-α, C-Reactive Protein and adipokines like adiponectin. Obese people have higher circulating concentrations of many inflammatory markers than lean people, and these are believed to play a role in causing insulin resistance and metabolic syndrome.

Keywords: Obesity; metabolic syndrome; inflammation

ÖZET Obezite, diyabet, kalp hastalığı, yüksek tansiyon ve bazı kanser türleri gibi birden fazla hastalık riskini artırdığı için ciddi bir halk sağlığı sorunu olarak kabul edilmektedir. Metabolik sendrom, intraabdominal obezite, hiperglisemi ve hipertansiyon gibi çeşitli metabolik anormalliklerden oluşur. İnflamasyon, bağışıklık sisteminin doğal bir parçasıdır ve vücudun birçok zararlı, yabancı ve yıkıcı etkiye karşı doğal savunma tepkisidir. Ancak kendi dokularına karşı inflamasyon oluştuğunda kronik inflamasyon gelişir. Obezite düşük dereceli kronik inflamasyona neden olur. Yağ dokusu en önemli endokrin organlardan biridir ve İnterlökin-1, İnterlökin-6, Tümör Nekroz Faktörü-α, C-Reaktif Protein ve adiponektin gibi adipokinlerden oluşan birçok inflamatuar mediyatör salgılar. Obez insanlar, zayıf insanlara göre çok sayıda inflamatuar belirteçten daha yüksek dolaşım konsantrasyonuna sahiptir ve bunların insülin direncine ve metabolik sendroma neden olmada rol oynadığına inanılmaktadır.

Anahtar Kelimeler: Obezite; metabolik sendrom; inflamasyon

OBESITY

The increasing prevalence of obesity is a major health problem not only in developed but also in developing countries. In developing nations, the prevalence of obesity has tripled as a result of the adoption of a western diet and lifestyle.[1] An increasing number of people all over the world, including those in Turkey, are adopting sedentary lifestyles that are based on fast food and lack regular physical activity. Worldwide, obesity and its complications are becoming an epidemic. Obesity is recognized as a serious public health problem, as it increases the risks of multiple diseases. Obesity has been linked to at least 300,000 deaths in the United States annually.[2,3]

The World Health Organization (WHO) defines obesity as an abnormal or excessive accumulation of fat in adipose tissue. Body mass index (BMI) is commonly used to classify overweight and obesity in adults. It is calculated as a person's weight in kilo-

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grams divided by the square of his height in meters (kg/m^2). In adults, overweight is defined as a BMI between 25-29 kg/m^2 and obesity is defined as a BMI greater than or equal to 30 kg/m^2. Classification of obesity according to Body Mass Index (World Health Organization) is shown in Table 1.[4,5]

TABLE 1: WHO classification of weight status.

Weight Status	Body Mass Index (BMI), kg/m^2
Severely underweight	<16.5
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	25-29.9
Obesity	>30
Obesity class I	30-34.9
Obesity class II	35-39.9
Obesity class III	≥40

METABOLIC SYNDROME

Metabolic syndrome was first described in 1988 and is also called Syndrome X, Reaven syndrome and insulin resistance syndrome.[6] Metabolic syndrome has been subject to several definitions since its initial description, and it consists of several metabolic abnormalities. Obese people frequently exhibit metabolic syndrome symptoms like insulin resistance, high blood pressure, and dyslipidemia. It can present in several forms, based on the combination of the various syndrome components. As most recently defined by the US National Heart, Lung and Blood Institute and American Heart Association Consensus Statement, metabolic syndrome is a constellation of 3 or more risk factors, which include abdominal obesity, high triglycerides, low- and high-density lipoprotein cholesterol, high blood pressure, and elevated fasting blood glucose (Table 2).

TABLE 2: Clinical diagnosis of the metabolic syndrome.

Risk Factors	Defining Level
Abdominal obesity, given as waist circumference	
Men	>102 cm (>40 in)
Women	>88 cm (>35 in)
Triglycerides	≥150 mg/dL
HDL cholesterol	
Men	<40 mg/dL
Women	<50 mg/dL
Blood pressure	≥130/≥85 mmHg
Fasting glucose	≥110 mg/dL

BMI: Body mass index; HDL: High-density lipoprotein.

Metabolic syndrome is closely associated with obesity and inactivity, and its prevalence is increasing rapidly all over the world. However, the beginning of the process is still unknown. Although the pathogenesis of metabolic syndrome has not been completely understood, insulin resistance and chronic low-grade inflammation derived from central obesity are the most widely accepted as underlying pathophysiology. People with metabolic syndrome also have a higher risk of many chronic diseases like type 2 diabetes, atherosclerosis, some types of cancer, and respiratory problems.[7]

METABOLIC SYNDROME, OBESITY AND INFLAMMATION

The adipose tissue is made of a large number of adipocytes, other non-fat cells, connective tissue matrix, vascular and neural tissues. The non-adipocytes cellular component includes inflammatory cells (macrophages), immune cells, preadipocytes and fibroblasts.

Adipose tissue is important for storing energy in the form of lipids, and it also protects the body from heat and cold by acting as an important cushion. In addition to serving a passive role, adipose tissue is a significant endocrine organ that generates hormones like leptin, estrogen, resistin, and the cytokine tumor necrosis factor alpha (TNF-α).[8]

Visceral adipose tissue (VAT) refers to the fat lining internal organs, whereas subcutaneous adipose tissue (SAT) refers to the fat under the skin. The placement of two adipose tissues in the body differs significantly anatomically. VAT primarily exists in the mesentery and omentum and drains to the liver directly through the portal circulation. Total body fat (TBF), VAT, and SAT normally rise as a kid becomes older during growth, while males and females show different trends.[9] High levels of TBF, VAT, and SAT are linked to a number of health hazards, such as insulin resistance, hepatic steatosis, metabolic syndrome, and hypertension.

The primary factor that determines insulin resistance is visceral fat, and numerous researchers have found associations between excessive visceral fat and an exaggerated inflammatory state.[10] Instead of extra-abdominal (subcutaneous) fat obesity, there is a strong correlation between increased morbidity and visceral (intra-abdominal) obesity.

Inflammation is a natural defense response of the body against all kinds of foreign and destructive factors,

whether immunological or non-immunological. From changes in body temperature to local vascular responses, inflammatory factors like chemokines, cytokines, eicosanoids, and biogenic amines play a significant role in various biological processes. This biological response first eliminates the causes of cell damage, then removes necrotic cells and tissues resulting from cell damage and initiates tissue healing. If inflammation develops against one's own tissues and cannot be adequately controlled, it turns into chronic inflammation, which reveals various pathologies including metabolic syndrome, diabetes, obesity and cancer.[11]

In infectious diseases, pathogens often initiate the first signal of classical inflammatory responses. However, the signals that initiate obesity induced inflammation are not clear. It is extremely important to understand the cellular mechanisms between the metabolic abnormalities that occur in the metabolic syndrome and obesity. The link between obesity and inflammation accelerated with the realization that cytokines are overexpressed in obesity.[12] Adipose tissue includes adipocytes, stromal preadipocytes, immune cells, and endothelial cells. Adipokines, including TNF-a, interleukins, plasminogen-activator inhibitor type 1, leptin, resistin, and adiponectin are a family of hormones and cytokines with both pro- and anti-inflammatory effects that are secreted by adipose tissue. In the case of obesity, adipocyte hypertrophy and hyperplasia occur in adipose tissue.[13]

With obesity and progressive adipocyte growth, blood flow to adipocytes decreases and hypoxia occurs.[14] Hypoxia causes necrosis and macrophage infiltration in adipose tissue. This is thought to lead to overproduction of proinflammatory factors such as inflammatory chemokines.

Adipokines have pro-and anti-inflammatory properties and play a critical role between systemic metabolism and immune function. In individuals with normal metabolic status, there is a balance of pro- and anti-inflammatory adipokines. When adipose tissue decreases in the body, proinflammatory adipokines decline and anti-inflammatory adipokines increase, which contributes to the suppression of immune function. As adipose tissue grows during the progression of obesity, this balance changes to favor proinflammatory mediators. In other words, while the levels of tumor necrosis factor-alpha and interleukin 6 (IL-6), which are known as proinflammatory adipokines, increase in obesity, the levels of the anti-inflammatory adipokine adiponectin decrease.[15] The cytokine concentration changes between the lean and the obese states are shown in Figure 1.

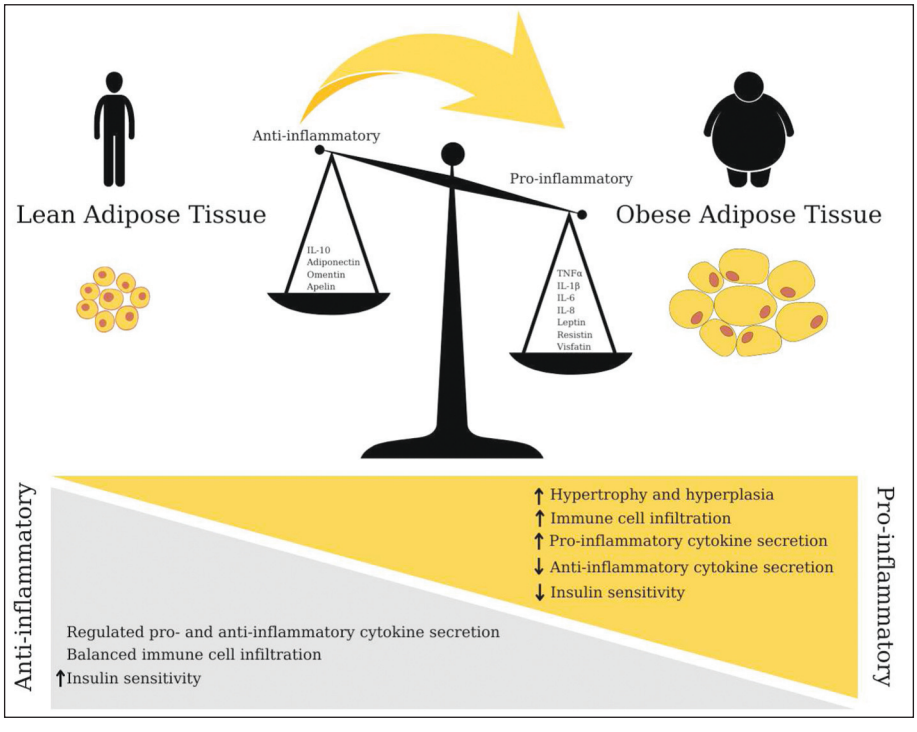


FIGURE 1: Transition from the lean/healthy state to the obese/pathological state.

TNF-a: Tumor necrosis factor alpha is a cytokine that has several functions in the body's various systems. Initially identified as a cytokine that contributes to the necrosis of tumors, TNF-α has more recently been shown to perform crucial additional roles as a pathogenic component of some inflammatory and autoimmune diseases.[16] The marker TNF-α is involved in the inflammatory process, and able to either directly stimulate or inhibit the functions of various homeostatic mechanisms which include activating lipolysis, inhibiting gluconeogenesis, and increasing vascular permeability. An increase in TNF-α increases the secretion of other proinflammatory cytokines such as IL-6 and reduces the secretion of anti-inflammatory cytokines such as adiponectin.[17]

Evidence suggests that TNF-α induces adipocyte apoptosis and increases insulin resistance by inhibition of the insulin receptor substrate 1 signaling pathway.[18] Circulating levels of TNF-α, a pro-inflammatory cytokine with numerous effects on adipose tissue, increase with obesity and decrease with weight loss.

IL-6: Pro-inflammatory cytokines play important roles in inflammation and among them, IL-6 is a major player in chronic inflammatory diseases, autoimmune diseases, and cancer. The main source of circulating IL-6 is macrophages that have infiltrated visceral or abdominal white adipose tissue. IL-6 is crucial for controlling inflammation and the balance of the entire body's energy supply. IL-6 produces inflammatory effects by inducing the transcription of factors in multiple pathways of inflammation. These may originate with protein kinase C, cAMP/ protein kinase A, and the release of calcium. Studies conducted *in vivo* and *in vitro* have shown that IL-6 can inhibit lipoprotein lipase activity. Additionally, the IL-6 receptor is present in a number of brain areas, including the hypothalamus, which regulates hunger and caloric intake.[19]

Adiponectin: Adiponectin is a cytokine, that is almost exclusively produced in adipose tissue and is highly expressed by lean, healthy people. Adiponectin levels, however, have been found to decrease in pathological diseases like coronary artery disease (CAD), Diabetes Mellitus, and hypertension.[20] TNF-α and IL-6 both inhibit the synthesis of adiponectin, while adiponectin suppresses the expression of TNF-α in adipocytes.[21] Plasma adiponectin levels are significantly lower in obese subjects compared with non-obese subjects. Adiponectin regulates lipid and glucose metabolism, increases insulin sensibility, and protects against chronic inflammation.[22] Many studies have shown that hypoadiponectinemia plays an important role in the development of insulin resistance, hyperinsulinemia, and type 2 diabetes.[23]

As a result, obesity is a complex disease involving an excessive amount of body fat. Obesity isn't just a cosmetic concern. It's a medical problem that increases the risk of other diseases and health problems. Obesity predisposes to a pro-inflammatory state via increased inflammatory mediators IL-6 and TNF-α, and reduced levels of adiponectin, which has totally anti-inflammatory function. The proinflammatory status of adipose tissue contributes to a chronic low-grade inflammation and it is considered a strong risk factor for developing many diseases including, metabolic syndrome, diabetes, cardiovascular diseases, and cancer.

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Identification of Endocrine Disruptors Via *in vitro* Diagnostic Tests: Current Methods and Future Aspects

in vitro Diyagnostik Testlerle Endokrin Bozucuların Tanımlanması: Mevcut Yöntemler ve Gelecek Beklentileri

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ABSTRACT Endocrine disruptor chemicals (EDCs) and their metabolites can interfere with the endocrine system of the organisms. EDCs can be classified as natural and synthetic chemicals. EDCs can enter the body's airways, skin, placental, and breastfeeding according to their chemical properties. diverse cellular functions and alter the functions of the endocrine system, nervous system, reproduction system, and immune system and cause adverse health effects on the metabolism of the organism. Metabolic-disrupting chemicals, neurological disruptors, reproductive disruptors, vascular disruptors, and immune disruptors molecules can be classified under the subgroup of EDCs and cause various diseases and disorders. All these chemicals can mimic the body's natural molecules can act as receptor antagonists or agonist molecules in many cellular major metabolic pathways, are involved in the energy production and utilization of the organisms, epigenetic alteration, signal transduction, receptor expression, hormone clearance, distribution, synthesis, transport and are involved in biochemical and physiological processes. Nowadays, EDCs can be detected by HPLC and gas chromatography methods. These exogenous compounds and their metabolites concentration must be determined via *in vitro* diagnostic assays. Determination of EDC via *in vitro* diagnostic testing, and detection in a normal routine biochemistry laboratory in the body fluids for instance blood, serum, plasma, urine, tear, spinal fluid, saliva, smear, amniotic fluid, cervical mucus, sperm, wound leakages, liquid biopsies, follicular fluid, pleural effusion, and sweat will be solve many health problems and opens the door of protect consumers from unhealthy cosmetic, and brings the discussions of water, food, air safety managements and standards.

Keywords: *in vitro* diagnostics; the imagination of future biochemistry; endocrine disruptor chemicals; metabolic-disrupting chemicals; metabolism

ÖZET Endokrin bozucu kimyasallar (EBK'ler) ve bunların metabolitleri, organizmaların endokrin sistemine müdahale edebilir. EBK'ler doğal ve sentetik kimyasallar olarak sınıflandırılabilir. EBK'ler kimyasal özelliklerine göre vücudun hava yollarından, deriden, plasentadan ve anneden süt emzirme yolu ile girebilir. EBK'ler endokrin sistem, sinir sistemi, üreme sistemi ve bağışıklık sisteminin fonksiyonlarını değiştirerek organizmanın metabolizması üzerinde olumsuz sağlık etkilerine neden olur. Metabolik bozucu kimyasallar, nörolojik sistem bozucular, üreme sağlığı bozucuları, damar sağlığı bozucuları ve bağışıklık sistemi bozucu moleküller ise EBK'ler alt grubu altında sınıflandırılabilir ve çeşitli hastalık ve bozukluklara neden olur. Tüm bu kimyasallar vücudun doğal moleküllerini taklit edebilir, birçok hücresel ana metabolik yolda reseptör antagonistleri veya agonist moleküller olarak davranabilirler, organizmaların enerji üretimi ve kullanımı, epigenetik değişim, sinyal iletimi, reseptör ekspresyonu, hormon klirensi, dağılımı, sentez, taşıma, biyokimyasal ve fizyolojik süreçlerde yer alırlar. Günümüzde EBL'ler HPLC ve gaz kromatografi yöntemleri ile saptanabilmektedir. Bu eksojen bileşikler ve bunların metabolitlerinin konsantrasyonu, *in vitro* diyagnostik yöntemleri ile belirlenmelidir. EBK'nin *in vitro* diyagnostik test yoluyla belirlenmesi ve kan, serum, plazma, idrar, gözyaşı, omurilik sıvısı, tükürük, smear, amniyotik sıvı, servikal mukus, sperm, yara sızıntıları, sıvı biyopsiler, foliküler sıvı, plevral efüzyon ve ter gibi vücut sıvılarında normal bir rutin biyokimya laboratuvarında saptanması, birçok sağlık sorununu çözecek ve tüketicileri sağlıksız kozmetikten korumanın kapısını aralayacak ve su, gıda, hava güvenliği yönetimleri ve standartları tartışmalarını beraberinde getirecektir.

Anahtar Kelimeler: *in vitro* diyagnostik; geleceğin biyokimyasını hayal etmek; endokrin bozucu kimyasallar; metabolizma bozucu kimyasallar; metabolizma

in vitro diagnostic assays have key roles in current medical diagnosis and treatment and in managing disease by analyzing various kinds of body fluids for instance blood, serum, plasma, urine, tear, spinal fluid, saliva, smear, amniotic fluid, cervical mucus, sperm, wound leakages, liquid biopsies, follicular fluid, pleural effusion, and sweat.[1]

in vitro diagnostic tests are developed according to the characteristic properties of the fluids. The range of the marker characteristics is variable in the content of the different types of trace elements, minerals proteins, peptides, enzymes, hormones, minerals, vitamins, immunoglobulins, and their important marker functions are enabling the development of the design of *in vitro* diagnostic test to be performed with related complex system engineering technologies.[2] In general, *in vitro,* diagnostics are divided according to the basic characteristics of the tests, for instance, biochemical, molecular, microbiological, and immunological diagnostics.[3]

There are essential factors that must be examined and developed for nearly all types of biochemical analysis and one of them is sample preparation, analysis, readout, and results.[4] In novel technology, microfluidic chips for *in vitro* diagnostic tests have several on-disc unit operation technologies, for instance, reagent storage, sedimentation, filtration, valving, decanting, aliquoting, mixing, separation, serial dilution washing, and calibration.[5] The integration of the methods and miniaturized sensor technologies are playing an increasingly crucial role.[6] And the suggested methodologies must be reliable, inexpensive, and rapid diagnostic tools.[5]

Immunoassays have an irreplaceable and crucial role in the diagnosis of various diseases on the other hand these tests have high costs and low automation properties which are not suitable for point-of-care tests (POCTs). On the other hand, the integration of luminescence detection into immunoassays on microfluidic chips would decrease the costs and increase the performance in quantification sensitivity and specificity.[7] Measuring more than one parameter via multiplexing the tests, would add fast and inexpensive properties to multifluid *in vitro* assays.[8]

Nowadays, capillary-driven microfluidic chips for *in vitro* assays are designed especially for patient-centric sampling strategies and they have common properties, for instance, these devices are designed as user-friendly, biohazard-free, and biomarker-capturing properties with multiplexing capabilities and applicable to a patient-centric workflow. These features open the doors to e-doctor and e-health applications.[9]

All *in vitro* diagnostic cells are measuring cellular metabolic products.[10] These metabolic products are the sum of sequential biochemical-biological processes which are under the control of many molecules and molecular mechanisms that are changing according to the health and disease conditions to sustain life. The metabolic pathways occur in the cytosol, and mitochondria are many remain unchanged in animals, plants, fungi, and bacteria.[11] Similar pathways are the sequence of enzymes catalyzed reactions that are affected by the same regulatory molecules in similar ways for instance hormones, xenobiotics, drugs, various chemicals, toxins, stresses, endogenous and exogenous cellular signaling molecules, hormones, environmental factors, metabolic disorders, and endocrine-disrupting chemicals.[12-15]

ENDOCRINE DISRUPTING CHEMICALS

Endocrine-disrupting chemicals (EDCs) are a group of natural or non-natural man-made chemicals that are generally foreign to cells or living organisms that are accepted as xenobiotics and these molecules can mimic hormones in many ways. EDCs can be taken via food, water consumption, industrial sources, consumer products, cosmetics, airways, skin, placental, and breastfeeding. Exposure to these chemicals begins from embryonic life.[16] EDCs can be used in many industrial products which can disrupt the natural hormone synthesis, secretion, metabolism, various enzyme activities, metabolic signaling, trace elements and minerals, reproduction of organisms, degradation, and transportation.[17-20] These molecules have many adverse effects on health for instance these molecules can induce hormone-sensitive cancers, thyroid, diabetes, alteration of adrenal hormones, neurodevelopment, and neuroendocrine system, and cause obesity and/or affect female and male reproduction system developmental anomaly.[21] These molecules have structural and functional effects on cellular metabolic enzymes via directly activating or inhibiting them.[18-20,22] And regulation of metabolic pathways would change metabolic products level and affect cellular homeostasis and cause metabolic disturbances.[23] The integration of metabolic products would affect diverse cellular functions and alter the functions of the endocrine system and cause adverse health effects on the organism.[24] EDCs can change DNA methylation, histone modification, and microRNA expression, and cause epigenetic modifications.[25] EDCs may induce oxidative stress, and due to the increased oxidative stress, it is linked to numerous health effects on organisms. Inhibition of the metabolic control activities of enzymes may cause the reprogramming of oxidative stress

metabolic enzymes, synthetic pathway enzymes, and energy balance.[18-20,22,26] Exposure to EDCs are triggering many coupled reactions for the metabolism of these foreign molecules and is attributed to increased caloric intake, physical inactivity, sleep deficit, obesity, Diabetes Mellitus, non-alcoholic fatty liver disease, and aging.[27] These complex EDCs would induce many metabolic processes and influence many complex biochemical reactions and can lead to the synthesis, transport, binding, action, and metabolism of endogenous hormones. And it is well known that EDCs have long-term adverse effects on newborns, children, and adolescents causing the type 2 diabetes in adults. Bisphenol A, phthalates, chlordane compounds, parabens, and pesticides are accepted as diabetes-related EDCs.[28] It has been also investigated in a previous research study that exposure to various types of EDCs, for instance, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), phthalates, per-and poly-fluoroalkyl substances (PFAS) can increase the gestational Diabetes Mellitus (GDM).[29] The studies that follow the effects of EDCs on human health are confirming that the effects of these molecules are not only restricted to types of diabetes but have adverse effects on hypertension and cardiovascular diseases.[30]

METABOLISM DISRUPTING CHEMICALS

Most of what we know about metabolism-disrupting chemicals (MDCs) is these molecules are classified under the EDCs. MDC can induce various metabolic, changes in the organism that can be determined from the serum biochemistry parameters.[31,32] EDCs have a group of chemicals, and this group of chemicals is named MDCs largely belonging to the category that affects metabolism. MDCs are controlling food intake and energy metabolism by altering the expression of neuropeptides within the hypothalamic circuits.[33] MDCs can cause metabolic diseases, obesity, type 2 diabetes, liver lipid disorders, metabolic syndrome, hepatic steatosis, affect adipocyte differentiation, increase adipogenic commitment, increase adipocyte proliferation, increase lipid uptake, increase fatty acid synthesis, decrease fatty acid catabolism and fatty acid efflux.[34] MDCs have crucial roles in protein metabolism. These chemicals can induce the uptake of various amino acids that have important roles in major pathways of metabolism, for instance, glutamine, glycine, tyrosine, lysine, proline, threonine, serine succinate, and taurine.[35] These molecules have various metabolism-modulating effects on metabolism.[36-43] Nowadays, the majority of the research on metabolism-regulating molecules and health focuses on EDCs and their combined effects on metabolism-regulating enzymes or oxidative stress enzymes.[18-20,22,26,32] Glucose-6-phosphate dehydrogenase is one of the rate-limiting enzymes of the metabolism and its activity is also affected by various kinds of EDCs dependent on the exposure time and concentration.[18-20,22,26,32,44]

MEASUREMENT OF ENDOCRINE-DISRUPTING CHEMICALS

Measurement of the EDCs is very valuable for instance nonylphenols (NP), bisphenol A (BPA), and dimethyl phthalate (DMP) can be measured by high-performance liquid chromatography.[45] Wang and his friends developed and validated a novel method for the determination of EDCs;[36] 8 bisphenols, 2 antimicrobials, 5 benzophenones, and 14 phthalate metabolites with ultra-performance liquid chromatography coupled to tandem mass spectrophotometry (UPLC-MS/MS) from human serum.[46] Detection of polybrominated diphenyl ethers (PDBEs), per-and poly-fluoroalkyl substances (PFAS), BPA, and phthalates were done by using gas chromatography/isotope dilution high-resolution mass spectrophotometry and high-performance liquid chromatography-isotope dilution-tandem mass spectrophotometry.[47] By using chromatographic separations bisphenol A (BPA), bisphenol B (BPB), bisphenol S (BPS), bisphenol F (BFF), bisphenol Z (BPZ), bisphenol AP (BPAP), bisphenol P (BPP); bisphenol AF (BPAF), bisphenol C (BPC), bisphenol E (BPE), tetrachlorobisphenol A (TCBPA) tetrachlorobisphenol S (TBBPS), methylparaben (MEP), ethylparaben (EtP), butylparaben (BuP), heptyl paraben (HeP), benzyl paraben (BzP), propylparaben (PrP) 4-hydroxybenzoic acid (4-HB) were determined from human serum and human urine.[48] Eleven different classes of EDCs were identified from drinking water by using analytical methods for instance HPLC, LC-MS/MS, GC-FID, GC-ECD, and GC-MS.[49]

CONCLUSION

The number and type of EDCs (natural or man-made) are increasing due to novel industrial products. Therefore, exposure to these EDCs is increasing in wildlife and all living organisms and affecting their physiology and increasing the health risk. The current methods that are used for the determination of EDCs are HPLC, LC-MS/MS, GC-FID, GC-ECD, GC-MS, and other chromatographic methodologies are time-consuming and very expensive. We need novel *in vitro* diagnostic tests to measure EDCs in our serum, blood, skin, etc. to reduce exposure to these chemicals and to support our body's detoxifica-

tion systems and protect ourselves from these pollutants and toxins. These *in vitro* diagnostic tests that analyze the samples must be rapid, economical, and easy to use which can analyze the health status of the human and all other living organisms due to exposure to different types of EDCs. Our world is polluted very fast and affecting all living organisms' life. We must hurry to protect our world and save the environment. All laboratory instruments and their chemicals must be green, protect nature, and protect our planet. We must change our laboratory techniques, chemicals, and instruments to protect lives and to protect the environment. This must be our collective and individual responsibility.

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Inflammatory Response in Wound Healing and Tissue Repair

Yara İyileşmesi ve Doku Onarımında İnflamatuar Yanıt

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ABSTRACT Inflammation, which is the second phase of wound healing, can be defined as the immune response of the organism against tissue damage. Considering that most of the injuries that occur on the skin in the evolutionary process are contaminated wounds, inflammation comes into play as a defense mechanism to prevent infection. This process, in which many cell types such as platelets, neutrophils, macrophages, mast cells, lymphocytes, keratinocytes, and dendritic cells play an active and synergistic role, provides debridement of the wound area before the repair phase begins, while forming a defense mechanism against infectious agents, and also acts as a secondary causes tissue destruction. The effects of vasoactive amines, plasma proteases, arachidonic acid metabolites, free radicals and cytokines secreted from inflammatory cells cause clinical symptoms such as edema, temperature increase and pain in the wound area. Chemotactic inflammatory mediators secreted from the cells also ensure that cell types such as fibroblasts and keratinocytes are brought to the environment, which will enable the wound to be repaired, that is, to proceed to the proliferation stage. With the effect of growth factors secreted from many cell types in the environment, neovascularization, collagen synthesis (granulation tissue formation), matrix formation and thus wound repair occur. Fetal wound healing involves little inflammation, scarless regeneration, and complete restoration of dermal structures. It is known that some tissues such as oral mucosa heal with minimal inflammation and minimal scarring in adults. The inflammation process lasts for 1-3 days in a normal wound healing, the prolongation of this process appears as a "chronic wound" in the clinic. So why is there such a phase in wound healing? The answer to this question is hidden in the evolutionary process. Injuries that occurred in primitive times were animal bites, falls, and contaminated injuries in the form of injuries with stones and branches. It was not possible to clean these wounds in a hygienic environment and to protect them from infection with the help of antibiotics. In this case, the organism had to remove dead cells, infectious agents and foreign bodies formed in the wound area with its own mechanisms. Inflammation was necessary at this stage. However, nowadays, more controlled healing of the wound can be achieved. The fact that the inflammation process is particularly long and intense, delaying the repair process has paved the way for many studies to take this process under control.

Keywords: Inflammation; wound healing; scar formation

ÖZET Yara iyileşmesinin 2. fazı olan inflamasyon organizmanın oluşan doku hasarına karşı verdiği immün yanıt olarak tanımlanabilir. Evrimsel süreçte deride oluşan yaralanmaların çok büyük oranda kontamine yaralar olduğu düşünüldüğünde inflamasyon enfeksiyondan korunma amaçlı bir savunma mekanizması olarak devreye girmektedir. Trombosit, nötrofil, makrofaj, mast hücresi, lenfosit, keratinosit ve dendritik hücre gibi pek çok hücre tipinin aktif olarak ve sinerjik bir şekilde rol oynadığı bu süreç onarım aşaması başlamadan önce yara bölgesinin debridmanını sağlar, enfeksiyöz ajanlara karşı savunma mekanizmasını oluştururken bir yandan da sekonder olarak dokuda yıkıma neden olur. İnflamatuar hücrelerden salgılanan vazoaktif aminler, plazma proteazları, araşidonik asit metabolitleri, serbest radikaller, sitokinlerin etkileri yara bölgesinde ödem, sıcaklık artışı ve ağrı gibi klinik semptomlara neden olurlar. Hücrelerden salgılanan kemotaktik inflamatuar mediatörler aynı zamanda yaranın onarımı yani proliferasyon aşamasına geçilmesini sağlayacak fibroblast ve keratinosit gibi hücre tiplerinin de ortama gelmesini sağlarlar. Ortamda bulunan pek çok hücre tipinden salgılanan growth faktörlerin etkisiyle neovaskülarizasyon, kollajen sentezi (granülasyon dokusu oluşumu), matriks oluşumu ve böylece yaranın onarımı gerçekleşir. Fetal yara iyileşmesinde çok az inflamasyon olur, skarsız bir rejenerasyon ve dermal yapılarda tam bir restorasyon oluşur. Yetişkin bireylerde de oral mukoza gibi bazı dokuların minimal inflamasyon ve minimal skarla iyileştiği bilinmektedir. İnflamasyon süreci normal bir yara iyileşmesinde 1-3 gün sürer, bu sürecin uzaması klinikte "kronik yara" olarak karşımıza çıkar. Öyleyse neden yara iyileşmesinde böyle bir faz vardır? Bu sorunun yanıtı evrimsel süreçte saklıdır. İlkel dönemlerde oluşan yaralanmalar hayvan ısırıkları, düşme, taş ve dallarla olan yaralanmalar şeklinde kontamine yaralanmalardı. Bu yaraların hijyenik bir ortamda temizlenmesi, antibiyotik yardımıyla enfeksiyondan korunması mümkün değildi. Bu durumda organizmanın yara bölgesinde oluşan ölü hücreleri, enfeksiyöz ajanları, yabancı cisimleri kendi mekanizmalarıyla ortamdan uzaklaştırması gerekiyordu. Bu aşamada inflamasyon gerekliydi. Ancak günümüzde yaranın daha kontrollü iyileşmesi sağlanabilmektedir. İnflamasyon sürecinin özellikle uzun ve yoğun olmasının onarım sürecini geciktirmesi, bu sürecin kontrol altına alınabilmesi yönünde pek çok çalışmanın yapılmasının önünü açmıştır.

Anahtar Kelimeler: İnflamasyon; yara iyileşmesi; skar oluşumu

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Acute inflammation can be defined as the body's response to trauma or damage caused by various reasons. Acute inflammation can be caused by bacterial or viral infection (e.g. acute respiratory distress syndrome (ARDS), tissue necrosis (acute myocardial infarction), trauma, radiation, burn or any foreign body. Inflammation is one of the stages of wound healing.

Wound healing can be defined as a drama in which many different cell types play a role. Mainly, it consists of 4 interconnected phases:

- Hemostasis
- Inflammation
- Proliferation
- Remodeling

Collagen and vascular damage that occurs when there is damage to the skin cause the initiation of intrinsic and extrinsic coagulation cascades. Platelets aggregate, trigger vasoconstriction to reduce blood loss, and a clot forms at the base of the wound. This clot creates a wound matrix, prepares an environment where different cells can migrate, almost a scaffold. Vasodilation occurs 5-10 minutes after vasoconstriction, platelets and leukocytes migrate into the matrix. Platelets degranulate, the complement cascade is activated. Many cytokines and growth factors released in the wound initiate a synergistic and communicating work between different cell groups.[1-14]

Local vasodilation, extravasation of blood and fluid into the extravascular space, and blockage of lymphatic drainage in the early stages of the injury process cause redness, swelling, and warmth, which are the main signs of inflammation. This acute inflammatory response usually lasts 24-48 hours.

Wound healing and regeneration is a complex process involving a functional inflammatory response. The inflammatory response initiates the repair process while fighting against infective pathogens. Inflammation, which is an evolutionarily developed process, strengthens the host reaction to tissue damage, has a critical role in maintaining tissue homeostasis, wound healing and tissue regeneration. The innate immune system and various cell types (neutrophils, macrophages, mast cells, various leukocytes) and a tightly regulated inflammatory process promote healthy wound healing and tissue regeneration (Figure 1).

However, in some chronic conditions, the irregular and prolonged inflammatory response may appear as a chronic wound that does not heal.

Cellular and systemic reactions resulting from bacterial infections, recurrent ischemia may result in hyperinflammation and pathological scar tissue formation.

In a normal wound healing, inflammation continues for 2-5 days. In the inflammatory phase, neutrophils and monocytes rapidly migrate to the damaged area. This phase

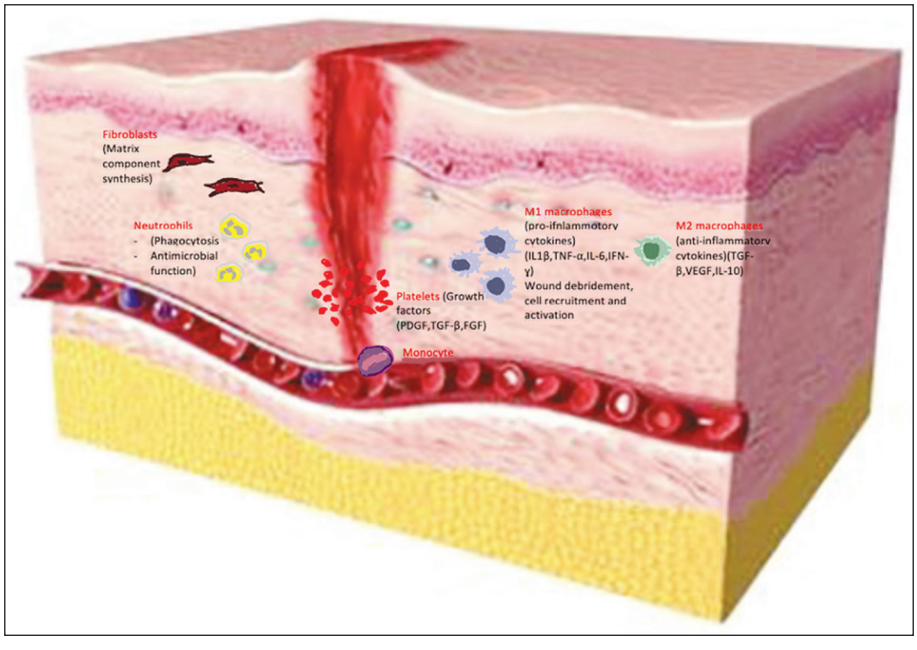


FIGURE 1: Many cell types play a role in inflammation. A weii-run inflammatory process is very important for wound healing.

is simultaneous with hemostasis and can be defined as the early phase of wound healing.

Inflammation; Its Role in Regeneration, and Repair

In case of tissue damage, innate and adaptive immune activation should be at an optimal level. Mammalian cells have the ability to detect cellular problems. When tissue is damaged, cells such as keratinocytes, macrophages, dendritic cells and mast cells are exposed to danger signals. These danger signals are divided into 2 groups: 1. Molecular patterns associated with injury (DAMPs), such as oxidized lipoproteins, DNA and RNA, secreted from cells under stress leading to necrosis. 2. Molecular patterns associated with pathogens. These are molecular patterns (PAMPs) that do not normally exist in the organism, such as bacterial polysaccharides, polynucleotides, non-enveloped viral RNA belonging to external pathogens.[3,4,15-18]

These danger signals are detected by pattern recognition receptors (PRRs) on the endosome on the cell surface or in the cytoplasm. These PRRs are Toll-like receptors (TLRs), RIG-1-like receptors (RLRs), and advanced glycosylation end-product receptors (RAGE). PRRs monitor cellular damage and initiate the appropriate response. Stimulation of these receptors causes activation of some signaling pathways. They activate transcriptional effectors such as nuclear factor kappa beta (NFKB) and mitogen activated protein kinases (MAPK), which trigger the expression of inflammatory cytokines. The secretion of these cytokines elicits the next phase of the immune response.[15]

Stimulation of Pattern Recognition Receptors (PRR) and inflammatory signals cause epigenetic changes that increase DNA accessibility. This is a critical cell adaptation. The cell can transform itself from its natural resting state into a cell that performs a special function (proliferate, migrate through the extracellular matrix, form new intercellular connections). That is, the cell at the injury site can undergo significant phenotypic changes. This transformation requires activation of silent genes in the basal state. This process is called “transflammation”.[15]

Accumulation of Professional Immune Cells

Inflammatory cytokines increase the expression of endothelial adhesion molecules [such as intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM-1)] and chemokines (such as MCP-1), thus attracting circulating immune cells such as neutrophils and monocytes in that region. The cytoskeletal structure of neutrophils that adhere to the endothelium and are exposed to chemokines changes and the neutrophil leaves the vessel. Extravasated neutrophils move from the region where the chemokines are less to the region where the chemokines are higher. The chemotactic factors secreted from mast cells during hemostasis provide the collection of neutrophils and monocytes in the tissue. Tumor Necrosis Factor (TNF), histamine, proteases, leukotrienes (LTs) and cytokines (interleukins) released from mast cells are other signals required for leukocyte recruitment. Growth factors such as Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor Beta (TGF-ß) are also chemotactic for leukocytes. If the wound is not infected, neutrophils remain in the environment for 2-5 days. Neutrophils increase the inflammatory response by producing cytokines such as TNF-α, interleukin (IL)-1b, IL-6.[1-14]

In the early phase of injury, circulating monocytes aggregate at the wound site. Among the chemoattractants that allow monocytes to come to the wound site, monocyte chemoattractant protein 1 and macrophage inflammatory protein 1 can be counted. Collagen fragments, fibronectin fragments and thrombin, which are extracellular matrix degradation products, are also specific chemoattractants for monocytes. In the early stages of the inflammatory phase, the predominant cell is proinflammatory macrophages (M1 macrophages) that transform from monocytes. Macrophages are the most important cells in the inflammatory phase. Macrophages have a special importance in inflammation and regeneration in organ damage caused by various reasons (such as infection, autoimmune diseases, mechanical damage).[1-3,7-13]

When there is tissue damage, matrix metalloproteinases secreted from macrophages disrupt the integrity of the basement membrane and allow inflammatory cells to collect in that area. They also guide this aggregation by producing chemotactic factors. Macrophages also phagocytose cellular debris, invading organisms, neutrophils, and apoptotic cells. Pro-inflammatory mediators such as IL-1, IL-6, IL-12, TNF α, and inducible nitric oxide synthase (iNOS) secrete chemokines and provide more leukocytes to collect in the environment. That is, in the absence of macrophages, the early inflammatory response is damaged. Debridement of the wound is reduced, the exposure time to proinflammatory stimuli is prolonged and this causes insufficient tissue regeneration.

This entire process by monocyte/macrophages induces angiogenesis and granulation tissue formation.

Dendritic monocytes process foreign proteins, enabling T cells to activate adaptive immunity, thereby recruiting cytotoxic T cells and antibody-producing B cells.

Macrophages secrete chemotactic factors (e.g. Fibronectin) and allow fibroblasts to come to the wound site. The development of new blood vessels is dependent on the

gradient of angiogenic factors produced by hypoxic macrophages because either full oxygen or anoxic macrophages cannot produce these angiogenic factors. Macrophages can be called growth factor production factories; e.g. PDGF, FGF, VEGF, TGF-ß, TGF-α. These cytokines induce cell migration and proliferation, and matrix production.[1-3,7-13]

In addition, local tissue macrophages and monocytes transform into the wound repair phenotype (anti-inflammatory) (M2) at the final stage of repair. These macrophages respond to IL-10 and other inhibitory mediators in the local microenvironment, secrete anti-inflammatory mediators such as IL-10 and TGF-B1, suppress the immune system by secreting cell surface receptors such as PD-L2, thus causing inflammation to collateral cell death and damage to the healing process, they prevent it from happening.[4,7,11-13,17-20] The shift of lipid mediators from proinflammatory prostaglandins to anti-inflammatory lipoxins is essential for the transition from inflammation to resolution. Lipoxins inhibit the recruitment of neutrophils, activate the recruitment of monocytes, and initiate tissue remodeling by removing dead cells by monocytes. Resolvins and protectins, another class of lipid mediators, also play a fundamental role in the resolution of inflammation and initiation of repair.

Wound repair macrophages also secrete many growth factors: PDGF, IGF-1, VEGF-α. They activate cell proliferation, blood vessel development, and all these reduce hypoxia that develops after injury. Macrophages also activate the transformation of tissue fibroblasts into myofibroblasts by producing mediators such as TGF-ß1, thus ensuring wound contraction and synthesizing extracellular matrix components. The proliferation of many adjacent parenchymal and stromal cells is also activated by macrophages. If tissue damage is severe or chronic, macrophages can also activate stem cells and local progenitor cells.[4,7,11-13,17-20]

Sometimes, dysregulation in wound repair responses can lead to pathological fibrosis and scarring, and damage to normal tissue function.

The adaptive immune system gives a later but specific response via B and T cells. B cells not only secrete antibodies, they also produce various cytokines and growth factors, they make antigen presentation, they regulate the activation and differentiation of T cells, and they provide lymphoid organization. B lymphocytes are found in the wound and play a critical role in healing.[1,6,9,10,13,17,19]

In wound repair, T lymphocytes are not only growth factor producing cells, but also immunological effector cells. Deficiency of CD4 or CD8 T lymphocytes alters inflammatory cell infiltration and cytokine expression profile. T cells stay in the medium for a long time and their number increases, and the CD-4-CD-8 ratio changes are observed in chronic wounds. Foxp3-expressing T cells (Tregs) control the immune response and inhibit autoimmunity. There are numerous Tregs in the skin. Activated Tregs accumulate in the environment during injury, they reduce IFN-γ production and proinflammatory macrophage accumulation, and increase wound healing via the EGFR pathway.

Innate and adaptive immunity play a key role in wound healing, and when damaged, plasmacytoid dendritic cells (pDCs) infiltrate the wound together with neutrophils. pDCs are sensitive to nucleic acids arising in the wound and produce type 1 interferons (IFN α/ß) via TLR7- and TLR-9 dependent pathways, a process critical in inducing early inflammatory response and reepithelialization. Langerhans cells (LCs) are a special subgroup of epidermal dendritic cells, the first group of defensive, epidermal immune surveillance.[1,6,9,10,13,17,19]

In the early stages of wound healing, Langerhans cells increase in the wound. Unlike the well-known α, β, T cells, they express antigen recognition receptors (TCR), which consists of γ, δ T cell subgroups γ and a δ subunits. The γ and δ T cells in the epidermis are known as dendritic epidermal T cells (DETC). In the wound, these cells recognize and eliminate damaged keratinocytes, and stimulate the proliferation of healthy keratinocytes by secreting growth factors such as FGF-7, keratinocyte growth factor (KGF-1), and IGF-1. In the wound, both α and β, and γ and δ T cells actively produce IGF-1. At the same time, γ and δ T cells produce IFN-γ and this ameliorates the antimicrobial, antitumor effects of NK α and β T cells. A subset of γ, δ T cells also produces IL-17, and they release a defense molecule from epidermal keratinocytes.[4,10,13,17,18,21]

CHEMICAL MEDIATORS OF INFLAMMATION

Many chemicals play a role in the initiation and control of inflammation. Some are protagonists, some are antagonists. They can be grouped as follows:[5,22]

- Mast cell mediators
- Plasma proteases
- Plasma proteins of the coagulation system
- Arachidonic acid metabolites
- Free radicals
- Cytokines and growth factors

MAST CELL MEDIATORS

An important substance released from mast cell granules is histamine. By acting on histamine receptor 1, histamine causes dilatation of arterioles and increased permeability of venules. Serotonin and heparin are also secreted from mast cell granules. These increase the permeability of the venules. Heparin is also an anticoagulant and prevents the coagulation of large amounts of tissue fluid and blood components.[5]

KININS

Kinins are biologically active peptides. They are found in areas with tissue destruction. The most well-known is bradykinin, which is released from plasma proteins in damaged tissue by the plasma enzyme kallikrein. Its effects on microvessels are similar to those of histamine.[5]

COAGULATION SYSTEM

Normally, natural anticoagulant mechanisms protect against thrombotic complications. Inflammatory mechanisms shift towards coagulation activation. Inflammatory mediators can increase platelet count and reactivity, while inhibiting natural anticoagulant mechanisms. Similarly, coagulation can increase the inflammatory response through mediators released from platelets.[22]

ARACHIDONIC ACID METABOLITES

Prostaglandin (PG) and leukotrienes (LT) are two potent products of arachidonic acid secreted from cell membrane phospholipids. They are produced in all body cells with cell membrane damage. PG I2; PG D2, PG E2 and PG f2α cause vasodilation, and also increase vascular permeability and cause edema. PG E2 also has chemotactic activity and allows leukocytes to collect in the wound. PG E2 works synergistically with other inflammatory substances such as bradykinin and creates hyperalgesia by sensitizing pain receptors. Another arachidonic acid metabolite is the LT group. LT B4 is a potent chemotactic agent and induces neutrophil aggregation, while LT C4, LT D4 and LT E4 cause vasoconstriction and increase vascular permeability.[5]

FREE RADICALS

Infiltrating immune cells produce reactive oxygen and nitrogen molecules against pathogens, but these molecules also cause tissue damage. Free oxygen radicals are cytotoxic to pathogens but also have a locally destructive effect. However, low levels of radicals are important in cell signaling. For example, hydrogen peroxide formed in the vessel wall causes vasodilation and thus increases tissue perfusion. Similarly, superoxide anion is required for the transformation of somatic cells into pluripotent cells.[5,19]

CYTOKINES AND GROWTH FACTORS

It has many critical roles. For example, PDGF is chemotactic for monocytes, macrophages and neutrophils, and mitogenic for fibroblasts and smooth muscle cells. Many growth factors secreted from macrophages provide cell proliferation, angiogenesis, and extracellular matrix synthesis. For example, TGF-α increases keratinocyte migration and reepithelialization, TGF ß1, TGF ß2 and TGF ß3 increase fibroblast and endothelial cell migration, provide extracellular matrix deposition by fibroblasts during granulation tissue formation. While increased TGF ß1 increases scar formation, TGF ß3 has an antiscar effect.[5]

REDUCED INFLAMMATION AND BETTER WOUND HEALING

Wound healing in the adult results in fibrous scarring, but the fetal skin heals with scarless regeneration and complete restoration of normal dermal structure. There is very little inflammation in this healing. Scar formation has been observed in fetal wounds when inflammation is provoked. Another example of this situation is the oral mucosa, where injuries occur with minimal inflammation, and the wound heals quickly and usually with minimal scarring. When an excisional wound with similar characteristics was created on the skin and tongue, significantly fewer neutrophils, macrophages, T cells, and fewer cytokines IL-6, KC (cytokine-induced neutrophil-attracting chemokine;a murine functional homologous to human IL-8) were found in the wound on the tongue.[6]

INCREASED INFLAMMATION AND DAMAGED WOUND HEALING

If the capacity of the innate immune system is insufficient or its defensive function is limited, the adaptive immune system is activated and specific B and T cells are activated. If this process is too long or insufficient, it is passed to the chronic stage. This is what is seen in some heart diseases or rheumatoid arthritis. Chronic inflammation sometimes plays a leading role in persistent bacterial infections (Tuberculosis), ARDS, autoimmune diseases, inflammatory bowel diseases, atherosclerosis, neurodegenerative and metabolic hormonal diseases.[19]

Leukocytes, especially neutrophils, are prominent in the chronic wound. Neutrophils produce growth factors as well as enzymes that induce tissue damage. Neutrophil proteases such as elastase and cathepsin G can degrade ex-

tracellular matrix components, proteins such as clotting factor, complement, Ig and cytokine. In addition to forming the extracellular matrix support skeleton, it also affects the migration and proliferation functions of keratinocytes. Therefore, modification of the extracellular matrix by neutrophils may have important effects on epithelial repair. In addition, the inflammatory cytokines produced by these neutrophils cause more inflammatory cells to be recruited and activated, more proteases are released and tissue damage increases. This suggests that even in normally healing wounds, the destruction of neutrophils may negatively affect the repair.[1-6,8,9,18-20,23] In a study in mice, neutropenia was induced by antineutrophil antibodies, and neutropenia at the time of injury accelerated the epithelial healing of the wound, but did not change the dermal healing capacity.[6]

IMMUNE MUTANTS AND HEALING

Studies in mice with defects in specific immune cells and molecules have shown that inflammation is not a necessary prerequisite for effective tissue repair. Wound repair in 1 null mice (without macrophages and functioning neutrophils) was scar-free as in the fetus.[6] In another study, cutaneous wound healing was delayed in mice genetically deficient in secretory leukocyte protease inhibitor (SLPI). SLPI is expressed in the skin and mucosa during wound healing, has an anti-inflammatory effect, and antagonizes the synthesis of proinflammatory mediators by monocytes and macrophages. SLPI deficiency increased the inflammatory response, increased elastase activity in the wound bed, and aberrant healing.[24]

Another key mediator of wound healing is TGF-ß, a multifunctional cytokine that activates inflammatory cells. Intracellular signaling of TGF-ß is governed by Smad proteins. In mice lacking the Smad 3 gene, skin wound healing is accelerated, the rate of reepithelialization increases, and the inflammatory cell effect decreases.[1,3-7,9-11,13,17-21,23]

WHY INFLAMMATION?

Although wound inflammation is a protective mechanism, these mechanisms, which kill microorganisms and remove debris, delay wound healing. If so, why is there significant inflammation in the wound? Could this be because primitive humans and animals require an intense inflammatory response to survive in their environment? Typical injuries in these periods were caused by surrounding stones, rocks, biting. In this case, microbial contamination was almost guaranteed. The wound was exposed, there was no antibiotic, clean water, sterile surgical intervention. In this case, healing with delayed and abundant scarring was more acceptable than systemic infection. Therefore, controlling the inflammatory response may be the right approach for faster and scar-free healing of sterile surgical wounds. The aim is to reduce the destructive effects of inflammation without increasing the risk of wound infection.

After a successful inflammatory response comes the repair phase. This is mainly managed by macrophages. The shift of lipid mediators from proinflammatory prostaglandins to anti-inflammatory lipoxins is essential for the transition from inflammation to resolution. Lipoxins inhibit the recruitment of neutrophils and activate the recruitment of monocytes, monocytes remove dead cells and initiate tissue remodeling. Resolvins and protectins, another class of lipid mediators, and TGF-ß and growth factors produced by macrophages have a fundamental role in the initiation of inflammatory resolution and repair.[4,7,11-13,17-20]

If the acute inflammatory response is unable to eliminate the pathogen, the inflammatory process continues and acquires new features. Neutrophils are replaced by macrophages, T cells also come in the presence of infection. If this is insufficient, the chronic inflammatory period is entered, granulomas and tertiary lymphoid tissues appear.

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Inflammation and Carcinogenesis

İnflamasyon ve Karsinogenez

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ABSTRACT Cancer is not one disease. Recent findings suggest that polarized inflammation plays different roles during tumor development and progression. The complexity of its origins, cellular behaviours, and molecular features thwarted researchers' efforts to grasp its multifaceted biology. The complexity in cancer lies not only in its genetics, but also in its versatile responses to an environment in which it has to compete to survive and thrive. It has been difficult to directly ask how they acquire and utilize nutrients *in vivo*. Advances in clinical imaging show us how tumors adjust their metabolic profiles to adapt to local conditions, even exhibiting heterogeneity within the same tumor mass. There is an active and dynamic communication between a tumor and the surrounding microenvironment, including the local immune components. Probing this interplay led to the identification of checkpoint blockade approaches that are generating excitement in the clinic. Further searching out the layered functional interactions between cancer cells and the immune system may open up new access points that could be targeted. Genomics, metabolism, and microenvironment-are only three examples of how our growing capacity to measure, analyze, and capitalize on the complexity inherent to cancer appears to be turning the achievements to scientists' advantage. With increased understanding of the specific mechanisms that regulate cancer-associated inflammation, there will be further opportunities to alter the course of cancer with selective and targeted anti-inflammatory treatment. The ability to map out the complexities of not only the tumor itself but also the environment it resides in is expanding the scope of translational research and drug development from a tumor-centric targeted view to a system-wide perspective that takes into account dynamic host response and tumor microenvironment communication.

Keywords: Inflammation; carcinogenesis; DNA damage; tumor microenvironment; inflammatory mediators

ÖZET Kanser tek bir hastalık değildir. Son bulgular polarize inflamasyonun tümör gelişimi ve ilerlemesi sırasında farklı roller oynadığını göstermektedir. Kökenlerinin karmaşıklığı, hücresel davranışları ve moleküler özellikleri, araştırmacıların kanserin çok yönlü biyolojisini kavrama çabalarını zorlaştırmıştır. Kanserin karmaşıklığı, sadece genetik yapısından değil, aynı zamanda hayatta kalmak, gelişmek için rekabet etmek zorunda olduğu mikroçevresine verdiği çok yönlü tepkilerden kaynaklanmaktadır. Kanser hücrelerinin besinleri *in vivo* olarak nasıl elde ettiklerini ve kullandıklarını açıklamak oldukça zordur. Ancak klinik görüntülemedeki ilerlemeler, lokal koşullara bağlı olarak bize tümörlerin metabolik profillerinin nasıl uyum sağladığını göstermektedir. Aynı tümör içerisinde bile hücrelerin heterojen bir profil sağladığı görülmektedir. Bir tümör ile onu çevreleyen mikroçevre arasında, lokal immün bileşenleri de dahil olmak üzere aktif ve dinamik bir iletişim vardır. Bu etkileşimin araştırılması, klinikte heyecan yaratan kontrol noktalarının belirlenmesine yol açtı. Kanser hücreleri ile immün sistem arasındaki katmanlı fonksiyonel etkileşimlerin daha fazla araştırılması, hedef alınabilecek yeni noktaların bulunmasını sağlayabilir. Kanserin genomik yapısının, metabolizmasının anlaşılması ve mikroçevresinin değerlendirilebilmesi, kanser haritasının doğasında var olan karmaşıklığı ölçe bilmemizi ve analiz etmemizi sağlayarak, yeni tedavi stratejilerinin ortaya çıkmasına olanak sağlamıştır. Spesifik mekanizmaların anlaşılmasının artmasıyla birlikte kansere bağlı inflamasyonun düzenlenmesi, seçici, hedefe yönelik antiinflamatuar tedavilerle kanserin seyrinin değiştirilebilmesine fırsat sağlamaktadır. Sadece tümörün kendisinin değil, aynı zamanda içinde bulunduğu ortamın karmaşıklıklarını haritalandırma yeteneği, translasyonel araştırma ve ilaç geliştirmenin kapsamını arttırmaktadır. Dinamik konak tepkisini ve tümör mikroçevre iletişimini hesaba katan, bu perspektife yönelen tedavi protokolleri ortaya çıkacaktır.

Anahtar Kelimeler: İnflamasyon; karsinogenez; DNA hasarı; tümör mikroçevresi; inflamatuar mediyatörler

KAYNAK GÖSTERMEK İÇİN:
Sökmen S, Manoğlu B. Inflammation and carcinogenesis. In: Koçdor H, Pabuçcuoğlu A, Zihnioğlu F, eds. Inflammation and *in vitro* Diagnostics. 1st ed. Ankara: Türkiye Klinikleri; 2024. p.106-22.

Carcinogenesis is a multi-step process during which the cells undergo many severe changes. It is characterized by excessive proliferation followed by escape surveillance by the immune system and metastases. Malignant tumors are not lonely 'creatures', and a heightened understanding of tumor-host crosstalk is evidently warranted.[1] A malignant tumor exerts effects on the organism just as the organism's physiology exert effects on the tumor. In fact, the body's responses to the tumor may be beneficial (e.g., immune defenses) and/or detrimental (e.g., inflammation). The generation of cancer cells is caused by a wide array of genomic alterations, mainly due to acquired somatic mutations and environmental factors.[2] Genetic and epigenetic changes occurred in oncogenes and in tumor suppressor genes, which are the two broad categories of genes that are involved in carcinogenesis. DNA damage is considered to be the crucial cause of cancer. Hallmarks of cancer are common traits that govern the transformation of normal cells to cancer cells. Six hallmarks were defined in 2000:[3] (1) self-sufficiency in growth signals, (2)insensitivity to anti-growth signals,(3) evading appoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. Four new hallmarks were included in 2011:[4] (1) abnormal metabolic pathways, (2) evading the immune system, (3) genome instability, and (4) inflammation (Figures 1, 2). Hence, inflammation is recognized as a hallmark feature of cancer development and progression.

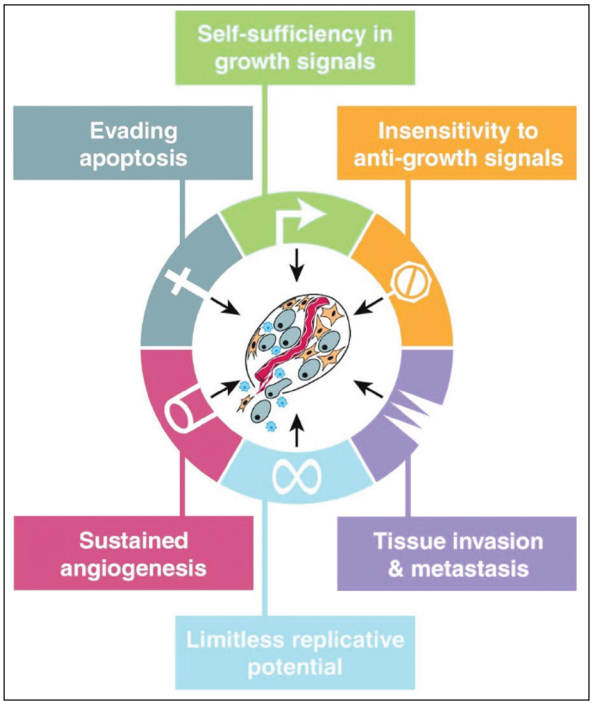


FIGURE 1: Hallmarks of carcinogenesis.[3]

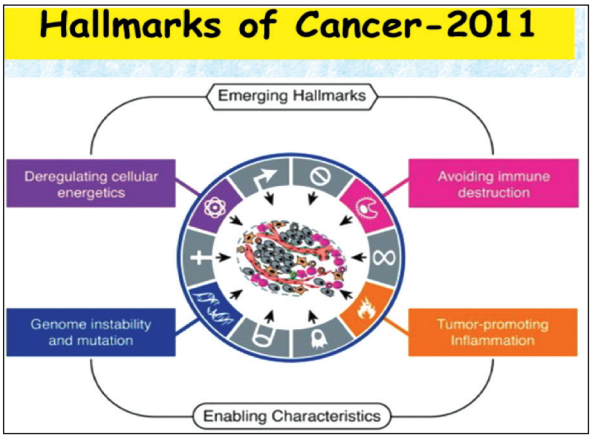


FIGURE 2: Hallmarks of cancer-updated: the next generation.[4]

The functional relationship between inflammation and cancer is not new. In 1863, Virchow hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation.[5] In 1918, Yamagiwa and Ichikawa, who showed that repeated painting of coal tar onto rabbits' ears caused carcinomas.[6-8] Inflammation is a local or systematic reaction from the cells in our immune system. There are different types of inflammation and that cells from other parts of the body are also involved. The cells that line our blood vessels and the cells that line our skin also have inflammatory responses, so we know that it's a more broad reaction that can involve cells throughout the body. Inflammation has a bad reputation, however, the truth is that we couldn't live without it. Inflammation is not strictly good or bad. It can be either, depending on how long the inflammation lasts and how much severe it is (Figure 3). While small flare-ups of inflammation are good since it's our immune system working to heal our bodies, but chronic inflammation can be an unwanted sign of something more relating such as rheumatoid arthritis, type 2 diabetes, Crohn's disease, or even solid cancer.[9-12] Examples of inflammation-related cancers are shown in (Table 1). Interestingly, at the same time, there are types of that help kill tumors: immunotherapies can capitalize on inflammatory processes to attack and treat cancer. Cancer's relationship with inflammation, ranging from how inflammation is involved in tumor growth to how inflammation can help fight cancer, is better understood in recent decades (Figure 4). As evident by Dr. Jim Allison's scientific discovery that led to immune check-

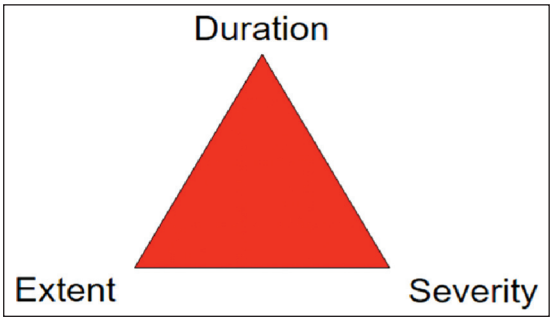


FIGURE 3: Three utmost important pillars of chronic inflammation-induced carcinogenesis.[2,20]

point therapy, we need to know details about the biology of inflammation and its association with cancer.

Inflammation is a vital component of tumour progression.[10,13-16] Many cancers arise from sites of infection, chronic irritation and inflammation. It is now becoming clear that the tumour microenvironment (TME), which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration.[17-19] In addition, inflammation involves interaction between various immune cells, inflammatory cells, chemokines, cytokines, and pro-inflammatory mediators ("Co-opted some of the signalling molecules of the innate immune system") (Table 2). It also plays a decisive role at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis (Figure 5). The view of cancer in relation to its host organism is evolving. Progressively, it is being viewed not as a single disease, but rather it is the sum of all events that will determine a specific disease outcome for a given individual, principally molded by both genetic and environmental factors.[20] These insights are fostering new anti-inflammatory therapeutic approaches to cancer.[21-24]

TABLE 1: Chronic inflammatory diseases vulnerable to tumorigenesis.[9]

Pathologic condition	Associated neoplasm(s)	Aetiologic agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibres, silica particles
Bronchitis	Lung carcinoma	Silica, asbestos, smoking (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic indwelling, urinary catheters
Gingivitis, lichen planus	Oral swquamous cell carcinoma	
Inflammatory bowel disease, Crohn's disease,	Colorectal carcinoma	
chronic ulcerative colitis		
Lichen sclerosus	Vulvar squamous cell carcinoma	
Chronic pancreatitis, hereditary pancreatitis	Pancreatic carcinoma	Alcholism, mutation in tryosinogen gene on Ch.7
Reflux oesophagitis, Barrett's oesophagus	Oesophageal carcinoma	Gactric acids
Staladenitis	Salivary gland carcinoma	
Sjögren syndrome, Hashimoto's thyroiditis	MALT lypmhoma	
Skin inflammation	Melanoma	Ultraviolet light
Cancers associated with infectious agents		
Opisthorchis, Cholanigitis	Cholanglosarcoma, colon carcinoma	Liver flukes (*Opisthorchis viverrini*), bile acids
Chronic cholecystitis	Gall bladder cancer	Bacteria, gall bladder stoner
Gastritis/ulcers	Gastric adenocarcinoma, MALT	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Mononucleosis	B-cell non-Hodgkin's lymphoma,	Epstein-Barr Virus
	Burkitis lymphoma	
AIDS	Non-Hodgkin's lymphoma,	Human immunodeficiency virus,
	squamous cell carcinomas, Kaposi's sarcoma	human herpesvirus type 8
Osteomyelitis	Skin carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease, chronic cervicitis	Ovarian carcinoma, cervical/anal carcinoma	Gonnorrhoea, chlamydia, human papillovirus
Chronic cystitis	Bladder, liver, rectal carcinoma,	Schistosomiasis
	follicular lymphoma of the spleen	

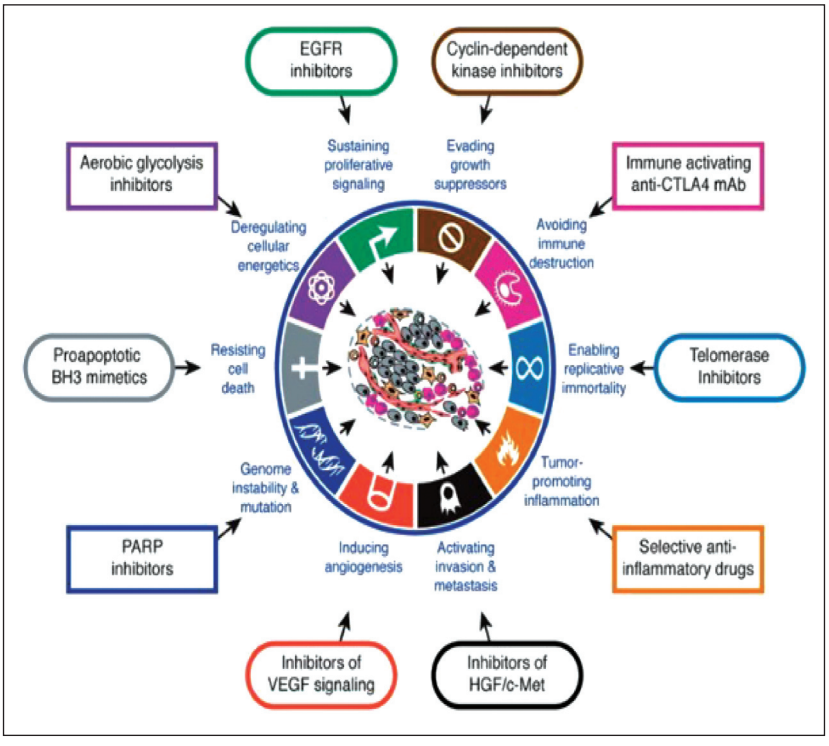


FIGURE 4: The interplay between inflammation and carcinogenesis and the schema to show that the niches we can use inflammation to gain therapeutic advances.[4]

TABLE 2: Major mediators of inflammation.[15]

Mediators	Source(s)	Main action(s)
Cell-derived		
Cytokines (TNF, IL-1, IL-6, IL-22…)	Macrophages, endothelial cells, T lymphocytes	Responsible for the systemic effects of inflammation Recruitment of neutrophils and monocytes
Chemokines	Leukocytes, activated macrophager	Chemotaxis
Reactive oxygen species	Leukocytes	Tissue damage
Nitric oxide	Macropgahes, endothelial cells	Recruitment of leukocytes
Leukotrienes	Leukocytes	Chemotaxis, vascular permeability
Plasma-derived		
Complement	Plasma (produces in liver)	Chemotaxis, other immune functions
Kinins	Plasma (produces in liver)	Vascular permeability
Proteases of the coagulation system an the fibrinolysis system	Plasma (produces in liver)	Recruitment of leukocytes, induce production of chemokines and nitric oxide

Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma, and DNA-damage-promoting agents, certainly potentiates and/or promotes neoplastic risk. Proliferating cells that sustain DNA damage and/or mutagenic assault (for example, initiated cells) continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumours act as wounds that fail to heal (Figure 6). The "normal" inflammatory response associated with wound healing is self-limiting. However, dysregulation of any of the converging key factors/steps leads to abnormalities, and ultimately pathogenesis, this seems to be the case during neoplastic progression **("dishomeostasis and maladaption")** (Figures 7, 8).

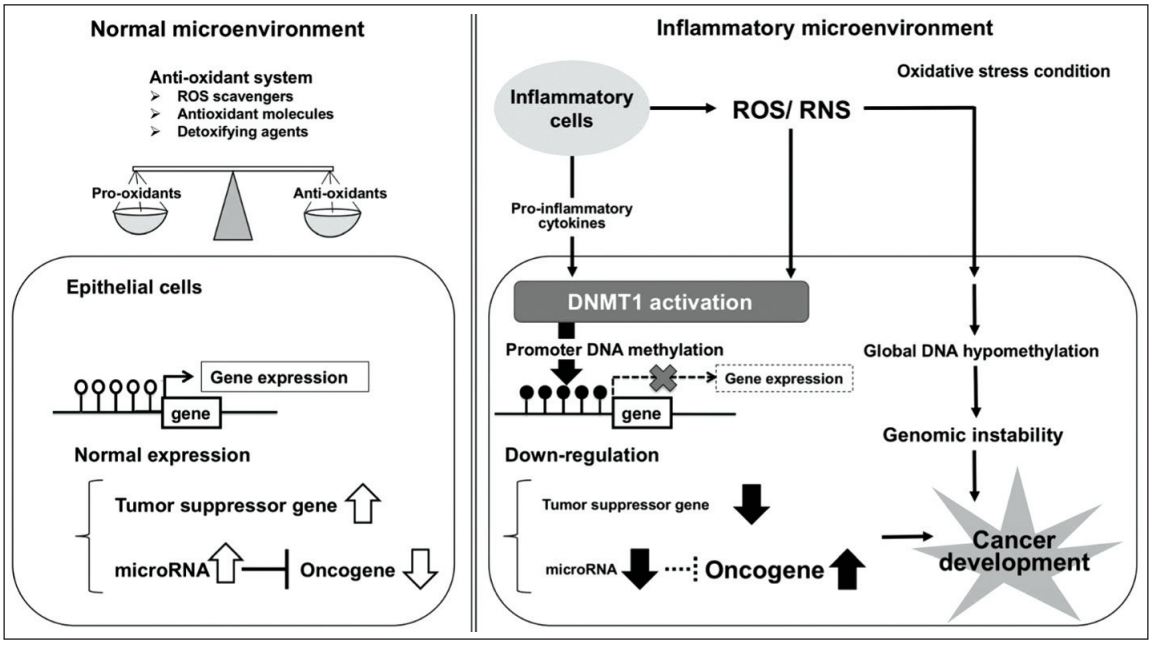


FIGURE 5: Epigenetic alterations under an inflammatory microenvironment.[16]

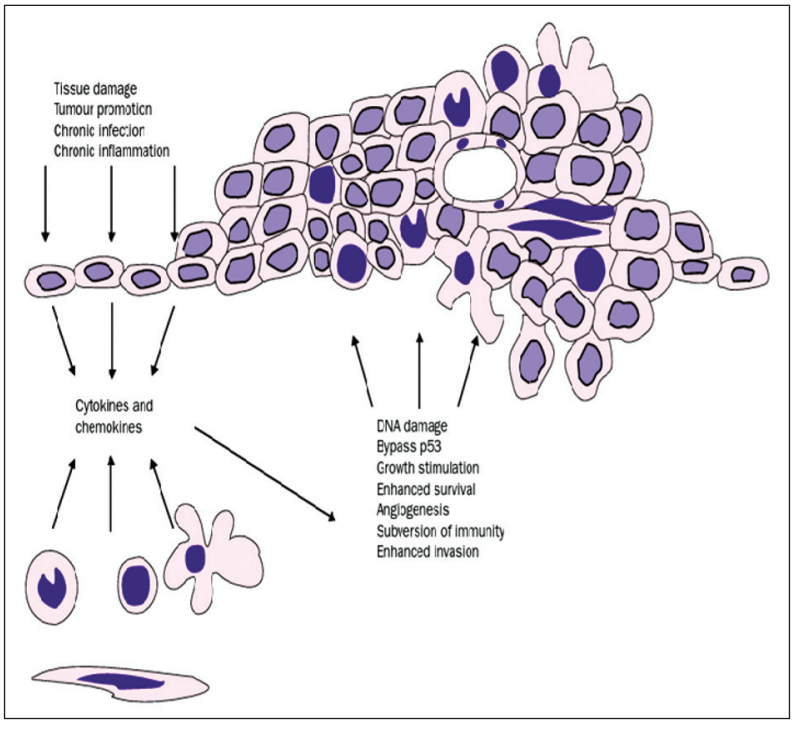


FIGURE 6: Chronic inflammation-induced carcinogenesis: tissue damage and chronic infection may stimulate cytokines and chemokines that contribute to malignant disease.[5]

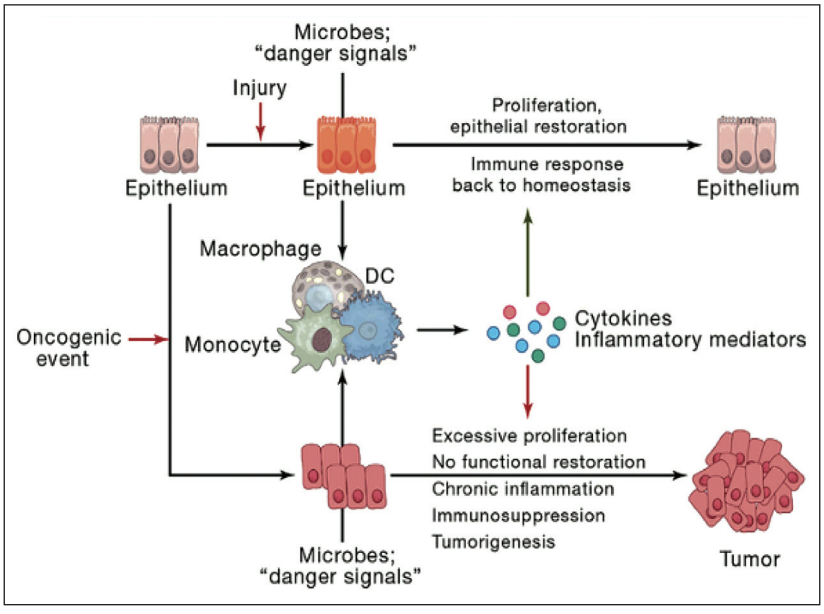


FIGURE 7: Evolutionary and functional differences and similarities between inflammation in cancer and inflammation during infection and tissue regeneration.[10]

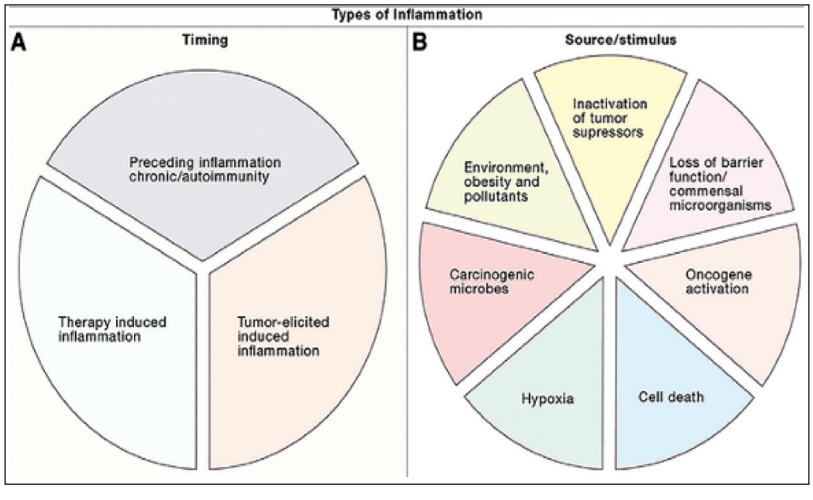


FIGURE 8: Types of Inflammation in Cancer: Different Timing and Different Inducers. **(A)** Cancer-associated inflammation can be induced at different time points of tumor development. It can precede carcinogenesis in form of autoimmunity or infection, can be induced by malignant cells or can be triggered by anti-cancer therapy. **(B)** Various cell intrinsic, host dependent or environmental factors can cause tumor-associated inflammation in different tumor types.[10]

INFLAMMATION AND NEOPLASTIC PROGRESSION

Inflammation predisposes to the development of cancer and promotes all stages of tumorigenesis. Peyton Rous was the first to recognize that cancers develop from "***sub-threshold neoplastic states***" caused by viral or chemical carcinogens that induce somatic changes. These states, now known as 'initiation', involve DNA alterations, are irreversible and can persist in otherwise normal tissue indefinitely until the occurence of a second type of stimulation ('promotion'). Functionally, many promoters, whether directly or indirectly, induce cell proliferation, recruit inflammatory cells, increase production of reactive oxygen species (ROS) leading to oxidative DNA damage, and reduce DNA repair. Cell death and/or repair programmes

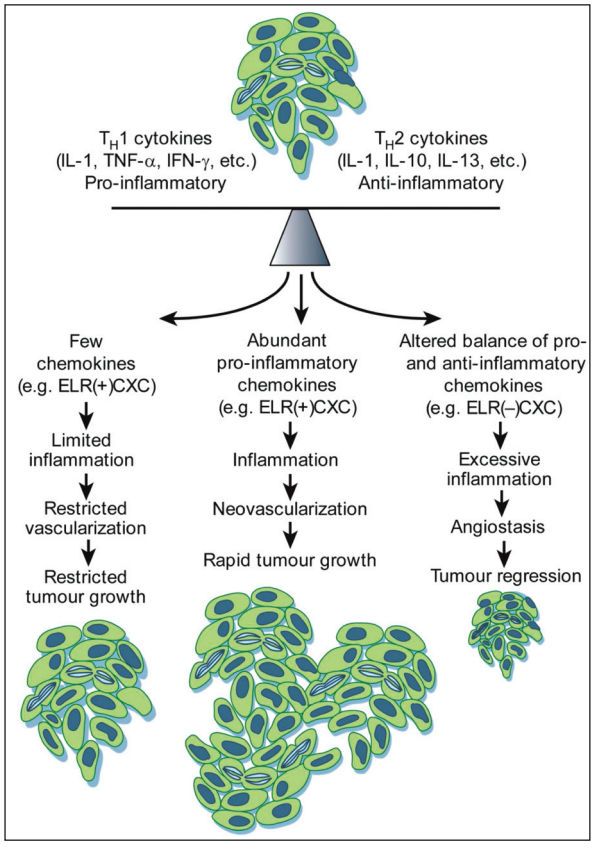


FIGURE 9: Cytokine and chemokine balances regulate neoplastic outcome.[9]

ocur in chronically inflammed tissues, thus resulting in DNA replication and proliferation of cells that have lost normal growth control. Under normal circumstances, the production of anti-inflammatory cytokines follows the pro-inflammatory cytokines closely (Figure 9). However, chronic inflammation seems to be due to persistence of the iniating factors or a failure of mechanisms required for resolving the inflammatory response. *Why does the inflammatory response to tumors persist?*

ROLE OF TUMOR-ASSOCIATED MACROPHAGES (TAM) IN CANCER RELATED INFLAMMATION[17,25-29]

Tumor cells produce various cytokines and chemokines that attract leucocytes. The inflammatory component of a developing neoplasm may include a diverse leucocyte population-all of which are capable of producing an assorted array of cytokines, cytotoxic mediators including ROS, serine and cysteine proteases, MMPs and membrane perforating agents, and soluble mediators of cell killing, such as TNF-alpha, interleukins (IL) and interferons (IF). Macrophages are a fundamental part of the innate defense mechanisms, which can promote specific immunity by inducing T-cell recruitment and activation. Despite this, their presence within the tumor microenvironment has been associated with enhanced tumor progression and shown to promote cancer cell growth and spread, angiogenesis, and immunosuppression. This paradoxical role macrophages in cancer finds an explanation in their functional plasticity, that may result in the polarized expression of either pro- or antitumoral functions. TAMs have a dual role in neoplasms-although they may kill neoplastic cells following activation by IL-2, IF and IL-12, TAMs produce a number of potent angiogenic and lymphangiogenic growth factors, cytokines and proteases, all of which are mediators that potentiate neoplastic progression. TAMs and tumor cells also produce IL-10, which effectively blunts the anti-tumor response by cytotoxic T cells. By placing TAMs at the centre of the recruitment and response to angiogenic and lymphangiogenic stimuli, they may foster the spread of tumors. Experimental and clinical evidence support a dual effect of inflammatory cells, macrophages in particular, in early as opposed to late phases of cancer development.[12] While high production of M1 inflammatory mediators (TNF, ROS) by inflammatory cells appears to support neoplastic transformation, phenotypic characterization of TAM in established cancers has invariably revealed their lack of M1 functions, along with the predominant expression of M2 properties. TAMs are not unique among inflammatoy cells in potentiation of neoplastic processes. Genetic and functional experiments indicate that neutrophils, mast cells, eosinophils and activated T lymphocytes also contribute to malignancies by releasing extra-cellular proteases, pro-angiogenic factors and chemokines (Table 3).

TABLE 3: Action of cytokines and chemokines which may facilitate cancer growth, invasion and metastasis.[5]

DNA damage via reactive oxygen
Inhibition of DNA repair via reactive oxygen
Functional inactivation of tumour suppressor genes
Autocrine/paracrine growth and survival factors for malignant cells
Induction of vascular permeavility and extravasation of fibrin/fibronectin
Tissue remodelling via induction/activation of matrix metalloproteinases
Control of tumour-cell migration, direct and indirect
Control of leucocyte infiltrate
Modulation of cell:cell adhesion molecules
Subversion of host immune responses
Stimulation of angiogenesis and angiogenic factor production
Resistance to cytotoxic drugs
Loss of androgen responsiveness

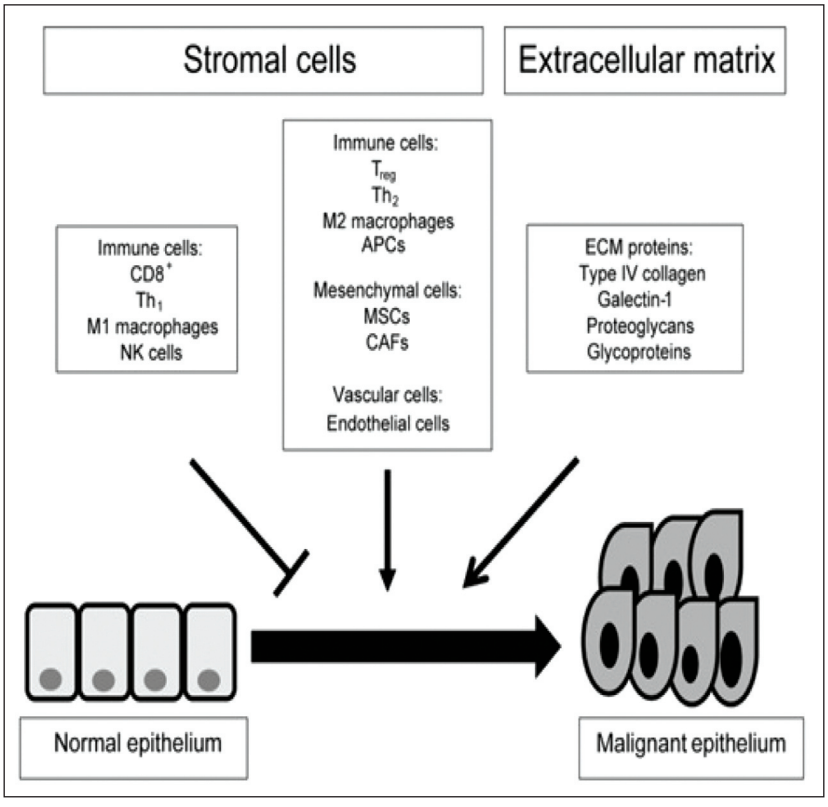


FIGURE 10: A top-down schematic view of the tumor microenvironment.[26]

Recent studies on the mechanisms of pro-tumorigenic inflammatory pathways in cancer have revealed that the pathways evolved to mediate immunity to infection and promote tissue homeostasis. The ***polarized inflammation*** can play various different roles during tumor iniation, growth, and progression. The inflammatuary cells, particularly macrophages, have a paradoxal "dual effect" in TME with distinct timing through the prior to or after iniation of tumorigenesis or might become evident only at the later stages of tumorigenesis. It is well-established that the TME contributes to cancer progression.[25,26,29-31] Inflammation, irrespectively its occurence in the context of a chronic inflammatory disease or in the appearance of a tumor-elicited smoldering inflammation, has a great effect on the composition of the TME and particularly on the plasticity of both tumor and stromal cells. Cancer cells as well as surrounding stromal and inflammatuary cells, organize in well-orchestrated interactions to form inflammatuary tumor microenvironment.[26] Stromal cells can be divided into mesenchymal, vascular, and immune. Signaling molecules secreted by the tumor corrupts these cells to create "activated" stroma (Figure 10). Equally, the extracellular matrix contributes to tumor development and invasion by forming a biologically active scaffold. Cells within the TME are highly functionally plastic, continuously changing their phenotypic and functional characteristics. Hence, a heightened understanding of tumor-host crosstalk is gradually evident which follows that cancer is "***not one disease***".[1,20] It is a multi-faceted complex-constructed biology showing problematic effects on organism's physiology, metabolism and immunity. This stochastic behavior of tumor cells challenge the development of effective anticancer treatment strategies and clinical trials, for studies do not catch the constant situation of all other confounding factors (**'ceteris paribus'**).[10,21] Other problems which present multiple scientific and logistical challenges for the development of cancer preventive agents targeting inflammation can be listed as:

1) Who are the subjects at greatest risk? (cohort selection),

2) Appropriate agent selection (target identification),

3) What are the best end-points?,

4) How to incorporate the temporal changes during carcinogenesis and inflammation into clinical trials that are relatively short?,

5) Tumor heterogeneity: cancer is not one disease even in the same target organ, cohort heterogeneity(pharmocogenetics, genetic-environmental changes and different stages of carcinogenesis),

6) Heterogeneity of the inflammatuary response(in different cancers, during carcinogenesis, different cohorts), and

7) Incomplete understanding of molecular pathogenesis, including inflammatuary response.

The concept of **"*bench to bedside*"** is widespread in translational research, but it has mostly been in a one-way street: bench science reveals a vulnerability of cancer cells, small molecules, or antibodies are developed, and then therapeutic value is assessed in clinical trials. Currently, the outcome of a trial can brand a translational concept as valid, whereas a failed one can be seen as nullifying any value of the preceding research efforts at the early drug development and preclinical stages. However, trials fail for a range of reasons, and the lack of success in one setting does not necessarily invalidate the promise of a translational idea. Rather, it could mean that we do not know enough of the underlying biology to effectively design trials for the right patient populations. The dramatic success of immunotherapy has propelled discussions of **"*reverse translational*"** research. It is clear that even when we see the achievements of immunotherapy, in many cases we don't fully understand why it is effective in some patients and not others.The large number of ongoing clinical trials, succesful or not, will yield data from which hypotheses can be generated and tested iteratively in the laboratory. This cycle doesn't just move the biology forward, but also informs trial design and patient selection.

The best example of using inflammation as an anticancer therapeutic opportunity comes from study of cancer risk among long-term users of aspirin and NSAIDs.[9,10,21,32] Much data indicates that use of these drugs reduces colon cancer risk by 40-50%. As the several cornerstone rules of engagement governing molecular and cellular mechanisms of tumor-promoting inflammation are constantly discovered, further development of anti-cancer therapies can be gradually distilled. Development of biomarker to monitor the treatment outcomes and toxicities would be very helpful in the translational medicinal research. In the fields of cancer, inflammation and immunity, this section aims to provide avenues for a more accurate dissection of oncogenesis, progression, and treatment in a physiological context. It has become clear now that the immune system can play significant pro- and anti-tumorigenic roles at all stages of the tumorigenesis.[33-35] This anti-tumorigenic role of the immune system is endogenous, i.e., being exerted normally in response to transformed and cancerous cells. One of the most promising recent developments in the field of cancer immunology is the successful implementation of various cancer immunotherapies, which use various approaches to redirect or hyperactivate the immune system toward the recognition, restraining, and killing of cancer cells. These approaches include immunological "checkpoint" blockade, immunization with cancer vaccines, neutralization of immunosuppressive cells, treatment with oncolytic viruses, or employing synthetic biology with bi-specific antibodies or cells with "chimeric antigen receptors".[10] The great promise and recent success of cancer immunotherapies also put a significant emphasis on studies of TME heterogeneity from the immunological standpoint of view. Terms of immunologically "hot" or "inflamed" TMEs are being coined primarily for tumors that have high levels of infiltrating T cells and increased presence of other components required for anti-tumor immune function, whereas immunologically "cold," "infiltration-excluded," "T cell excluded" and most importantly, "immunological desert" and "non-inflamed" terms are reserved for tumors that do not exhibit cellular and gene expression characteristics favorable for anti-tumor action primarily by T cells (**'*cancer-promoting inflammation*'**).[8-10,16,29] A vast body of studies now implicates inflammation and tissue repair immune responses to enhanced tumor incidence, growth, and progression. This evidence includes large cohorts on non-specific inhibition of inflammation with NSAIDs such as aspirin, reducing incidence and mortality in many cancers or specific inhibition of cytokines. Organ and site-specific chronic inflammation predisposes to cancer development at the same site. In the context of cancer,

TABLE 4: Chronic inflammation-induced solid organ carcinogenesis.[29]

Inflammatory bowel disease	Colorectal cancer
Gastric intestinal metaplasia	Gastric cancer
Barrett's esophagus	Esophageal cancer
Chronic hepatitis	Hepatocellular carcinoma
Chronic pancreatitis	Pancreatic cancer
Oral leukoplasia	Head/neck cancer
Atypical adenomatous hyperplasia	Non-small cell lung cancer
Ductal carcinoma in situ	Breast cancer
Prostatic intraepithelial neoplasia	Prostate cancer
Bladder dysplasia	Bladder cancer
Cervical dysplasia	Cervical cancer
Actinic keratoses	Skin cancer

in contrast to wound healing and infections that resolve after immune cell recruitment and epithelial cell proliferation, growing tumors present with persisting oncogene-derived stress, cell death, and microbial signals that altogether feed into a feed-forward loop of inflammation-induced signaling and inflammatory cell recruitment.

CHRONIC INFLAMMATION, INFECTION, AND AUTOIMMUNITY PRECEDING TUMOR FORMATION[2,6,9,10,14,26,31,33]

How are inflammatory cells co-opted into the neoplastic process? In order to deconstruct the roles and the mechanisms of action of inflammation and cancer, it is important to understand how inflammation in cancer is induced and maintained in the first place, both in terms of time and stimulus. A plausible hypothesis is that many malignancies arise from areas of infection and inflammation (Table 4), simply as part of the normal host response. Indeed, there is a growing body of evidence that many malignancies are initiated by infections-upwards of 15% of malignancies worldwide can be attributed to infections, a global total of 1.2 million cases per year. Persistent infections within the host induce chronic inflammation. Leukocytes and other phagocytic cells induce DNA damage in proliferating cells, through their generation of reactive oxygen and nitrogen species that are produced normally by these cells to fight infection. These species react to form peroxynitrite, a mutagenic agent. Hence, repeated tissue damage and regeneration of tissue, in the presence of highly reactive nitrogen and oxygen species released from inflammatory cells, interacts with DNA in proliferating epithelium resulting in permanent genomic alterations such as point mutations, deletions, or rearrangements.[12,13,36] Indeed, p53 mutations are seen at frequencies similar to those in tumours in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. The strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with inflammatory bowel diseases, for example, chronic ulcerative colitis and Crohn's disease (Figure 11). Hepatitis C infection in the liver predisposes to liver carcinoma, an increased risk of bladder and colon carcinoma is associated with schistosomiasis, whereas chronic *Helicobacter pylori* infection is the world's leading cause of stomach cancer. The Gram-negative bacterium *H. pylori* is established as a definite carcinogen for the development of gastric cancer-the second most common type of cancer globally-and DNA damage resulting from chronic inflammation is believed the mechanism. Exacerbating DNA damage induced by inflammatory cells is expression of macrophage migration inhibitory factor (MIF) from macrophages and T lymphocytes. MIF is a potent cytokine that overcomes p53 function by suppressing its transcriptional activity. Chronic bypass of p53 regulatory functions in infiltrated tissues can enhance proliferation and extend life span, while also creating an environment with a deficient response to DNA damage, amplifying accumulation of potential oncogenic mutations. Infectious viral agents, for example, DNA tumour viruses, may also directly transform cells by inserting active oncogenes into the host genome, although other mechanisms also are responsible. While many types of infectious agents are present in animals, only a subset of individuals infected with human papilloma virus, hepatitis B virus (HBV) or Epstein-Barr virus develop virus-associated malignancies. This may reflect immune suppression, the necessity of co-factors for promotion or the fact that a neoplasm can develop only if viral infection has targeted a pluripotent progenitor or stem cell. Such stem cells are typically low in abundance and located in regions of tissues

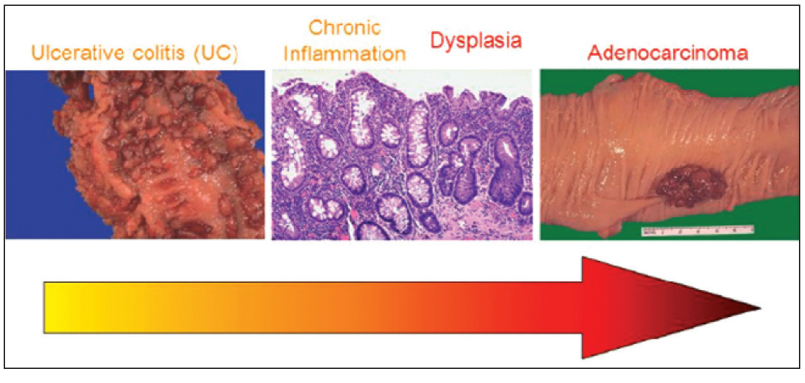


FIGURE 11: Colon cancer with the IBD has the worst prognosis.[33]

protected from agents that would otherwise harm them. In Rous sarcoma virus infections, inflammation is essential for tumour development and this requirement is mediated by factors such as TGF-b and other cytokines produced by the inflammatory cells. Epstein-Barr virus also causes sustained proliferation of B lymphocytes, which, when coupled with a secondary mutation, can result in neoplastic progression and malignant conversion to give rise to Burkett's lymphoma. The molecular mechanism behind the associated risk of hepatocellular carcinoma resulting from HBV and/or hepatitis C virus(HCV) infection is uncertain. Although there is evidence for clonal integration of viral DNA in tumours and surrounding parenchyma cells, there are no defined transforming sequences found within the viral genomes that can act as viral oncogenes. Moreover, there is no evidence to suggest that viral integration activates either a classical cellular oncogene or inactivates a cellular tumour suppressor gene. HCV core protein interacts with the signal transducer and activator of transcription 3 (STAT3) protein, a transcription factor involved in mediating cytokine signalling. This interaction induces sustained phosphorylation of a critical tyrosine residue, resulting in enhanced proliferation and upregulation of Bcl-xL and cyclin-D. Thus, chronic viral replication in hepatocytes may alter the local cytokine profile and the apoptotic or proliferative responses in infected cells, with an immune response to the viral proteins resulting in a state of chronic inflammation. Interestingly, a similar pathway involving inflammation, IL-6 and STAT3 is downstream of *H. pylori* in the generation of stomach cancer.

Around 15-20% of all cancer cases are preceded by infection, chronic inflammation, or autoimmunity at the same tissue or organ site. In such cases, inflammation that promotes cancer is induced and exists long before tumor formation. The most prominent examples include inflammatory bowel disease (IBD), chronic hepatitis, Helicobacter-induced gastritis, or schistostoma-induced bladder inflammation increasing the risk of colorectal cancer (CRC), liver cancer, stomach cancer, or bladder cancer, respectively. There are also various environmental factors

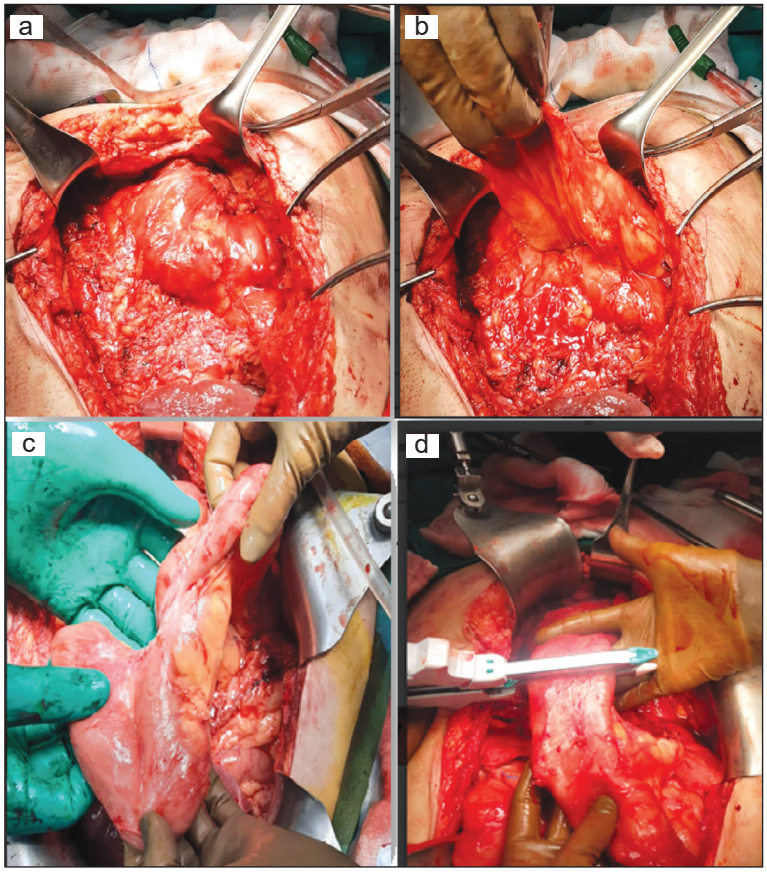


FIGURE 12: A patient with a long-term treated UC disease: chronic extensive inflammation, severe tissue damage, and a locally advanced rectosigmoidal carcinoma(Personal photodocumentation).

that predispose to and promote cancer acting in whole or in part via induction of chronic inflammation, albeit sometimes of low grade and intensity. In this case, inflammation might precede or accompany tumor development. With regard to the host, these factors might be systemic or site and organ specific. For example, inhalation of fine particles, tobacco smoke, and asbestos would primarily cause lung and airway inflammation and promote lung cancer and mesothelioma. On the other hand, low-grade inflammation induced by obesity, hyperglycemia, and excessive lipid accumulation is generally of systemic nature and, as a result, can promote or increase risk of many different cancers, including liver, pancreatic, colon, breast, and other malignancies. Type II diabetes, which has previously been considered as an independent risk factor for cancer, might be viewed as a part of obesity-induced inflammation and obesity-related tissue injury leading to cancer sequela. With the rapid emergence of the obesity epidemic in westernized countries, the exact mechanisms of how obesity and obesity-related inflammation promote tumor progression need to be determined in order to mitigate these life-threatening consequences of prevalent metabolic diseases.

The action of systemic inflammation can take place even during the late stages of tumor development, as exemplified by tobacco-smoke, obesity-, and bacterial-product-induced inflammation, which activate neutrophils and their extracellular trap formation function to promote breast cancer metastasis into the lungs. Inflammation and injury trigger cell turnover in tissues, thereby creating space for the outgrowth of malignant clones. Some of the immunological mechanisms Crohn's disease (CD) and ulcerative colitis (UC), as well as the expression of inflammatory mediators might be similar. However, UC which is associated with significant tissue damage and ulceration, followed by repetitive cycles of regeneration increases the risk of colorectal cancer (CRC) up to 5-8 times, whereas CD presented with transmural inflammation but not widespread epithelial damage increases the risk of CRC only 1.4 times (Figure 12). Therefore, whereas mere presence of inflammatory cytokines is needed for tumor iniation, cytokines collaborate with inflammation-induced tissue damage and regeneration. Clones with equal genetic alterations can possess different propensities for survival and outgrowth, depending on the TME these clones are placed into, illustrating the adaptive nature of the oncogenic process. Stressed and inflamed tissue might be conducive to stimulating tumor growth, whereas normal, unaffected tissue would block it. Similarly, the presence of inflammation can trigger outgrowth of dormant clones, both in the case of primary tumors and in distant metastasis. Some of the mechanisms responsible for this phenomenon might intersect with the ability of cytokines to promote survival and proliferation described above.

INFLAMMATION, TUMOR PROGRESSION, AND METASTASIS[8,10,11,19,25,27,28,32,35]

Studies on tumor initiation and growth were the first instrumental steps to demonstrate the critical role link between inflammation and cancer. However, understanding inflammatory mechanisms governing the metastatic process has the most eminent effect considering that over 90% of cancer-related deaths occur because of metastatic disease. Indeed, longitudinal clinical studies that support the role of inflammation in cancer mortality most likely underscore the role of inflammation in regulation of metastasis, rather than earlier stages of tumorigenesis. Prolonged aspirin usage reduces overall mortality, especially of gastrointestinal cancers, and distant metastasis. Experimental stimulation of resolution of inflammation or blocking inflammatory responses results in poor colonization and eradicates micrometastases. Metastasis is a highly inefficient process, and most of cells released from the primary tumor eventually die and do not form a distant metastasis. Therefore, any potential aid to get through the bottleneck would have a cardinal effect on the ''success'' of metastasis and potential patient survival. Given the apparent paucity of true metastatic models, some of the mechanisms of how inflammation might affect this process have only recently come to light. The process of metastasis starts with the invasion of cancerous cells away from epithelial layer into the neighboring tissues and acquisition of EMT(epithelial to mesenchymal transition) phenotype, at least temporarily. Although such epithelium-to-"fibroblast" cell transformation might be often incomplete and partial, it renders cancerous cells mobile and allows them to break through the basal membrane and invade the tissue and reach the lymphatics or blood vessels for further dissemination. In particular, cancer stem cells as opposed to "bulk" tumor cells are more efficient in their capacity to serve as metastatic seeds. Interestingly, cancer stem cells are also transcriptionally and functionally closer to mesenchymal cells than regular epithelial or bulk tumor cells, probably reflecting their existence in the niche rich in mesenchymal growth and differentiation factors. Inflammation influences cancer invasion, EMT, and cell migration on several levels. Cytokines, including TNF and IL-1b can directly affect expression of EMT-inducing transcription factors Twist and Slug. Moreover, IL-11 is involved in the recruitment

of tumor growth factor-b TGFb-driven and -producing fibroblasts, which supports tumor invasion and immune escape in the subset of colon cancer with the worst clinical prognosis. Similarly, in breast cancer, IL-11 within the TME acts on cancer cells to drive clonal selection of the most invasive and malignant clones. The recruitment of myeloid cells to the invasive edge of the tumor leads to production of matrix metalloproteinases (MMPs) and other enzymes, which remodel the extracellular matrix and facilitate cell migration. The same myeloid cells, particularly of granulocytic and monocytic lineage, sometimes referred to as myeloid-derived suppressor cells (MDSCs) also contribute to the suppression of anti-tumor responses. Increased accumulation of cytotoxic T and natural killer (NK) cells at the invasive margin of the primary tumor, along with decreased presence of myeloid cells as a part of the "cancer immunoscore" indeed correlates with better prognosis. Yet, it is not yet clear whether myeloid-induced immunosuppression is critical at the invasion stage or at preventing metastatic outgrowth. It is conceivable that macrophages play very diverse roles in immunosuppression, chemoattraction, and destruction of extracellular matrix as the invasion proceeds. Apart from that, tissue macrophages are critical cell types during the development of several organs and can assist cell migration and establishment of tissue architecture by producing factors directly influencing motility, phenotype, and positioning of epithelial and mesenchymal cells. In this regard, increased production of inflammatory entities causing enhanced recruitment of myeloid cell into the invading tumors might support tumor outgrowth via a variety of mechanisms that are evolutionarily reserved for the regulation of organogenesis during ontogeny. Cancer stem cells (CSCs) are considered essential for tumor metastasis and resistance to therapy, yet it becomes increasingly clear that the number and proportion of CSCs in the tumor are not constant, as they are for stem cells in normal tissue. Instead, various stimuli including prominent inflammatory signaling via transcription factors NF-kB and STAT3 in cancer cells can drive their stemness, increase the proportion of CSCs among the tumor cell population and thereby elevate the invasive potential. The same effects can originate from mesenchymal-cancer interactions, and mesenchymal and stromal cells can be regulated by inflammatory signals. Because metastatic spread typically occurs via lymphatics or blood stream, the processes of intravasation and extravasation are essential. These are mediated by the expression of adequate set of adhesion molecules and integrins, to allow heterotypic cell-cell interaction, adhesion, and movement. Inflammatory cytokines are potent inducers of integrins, selectins, and adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). The expression of exact and tissue specific adhesion molecules might also determine the organ-specific tropism of metastasis and therefore "flavors" of inflammation might potentially have an effect to which distant organ-particular tumor seed or type of cancer it will primarily spread to. The process of adhesion and extravasation can be further aided by inflammatory cells, such as monocytes and neutrophils which would precipitate the adherence of metastatic seeds, form complexes with cancer cells and mediate their adhesion and translocation throughout the vessel wall, as well as establishing and maintaining the metastatic niche. The oligocellular complexes between cancer cells themselves, or monocyte cancer cells, neutrophil-cancer cells also serve to protect these metastatic seeds from the immune surveillance at the most vulnerable moment away from established immunosuppressive microenvironment of the primary tumor. It is clear that inflammatory signals from obesity to microbes to tumor-specific inflammatory stimuli that enhance these cell-cell interactions increase the rate of metastasis. For example, IL-17-dependent activation of neutrophils drives breast cancer metastasis through cellular interactions in the circulation and possibly via establishment of a pre-metastatic niche, whereas lipopolysaccharide increases lung colonization and promotes tumor cell survival. Stimulation of inflammation by tobacco smoke, obesity, or microbial compounds also increases lung metastatic burden via induction of neutrophil activation and increased neutrophil-cancer cell interactions mediated by neutrophil extracellular traps (NETs), although it is not clear whether this process is regulated at the stage of spread or stage of dormancy. Although the process of metastatic growth from a smaller to a larger metastatic nodule is probably not much different from the growth of a primary tumor and the role of inflammation there, one of the roadblocks for metastasis that remains is the ability of single cells to take off and grow in the epithelial tissue of an origin different from the primary tumor. Because of that, initially after the spread and initial colonization some of the seeds remain at the dormant stage and their eventual outgrowth might be promoted by inflammatory cytokines and growth factors produced by immune cells. It remains to be determined across the different tumor types, whether the requirement of inflammatory and growth factors is the same for primary tumor growth and for metastatic outgrowth in different secondary organ sites.

INFLAMMATION AND CELL PLASTICITY WITHIN THE TUMOR MICROENVIRONMENT[8,10,11,25,30]

Tumor heterogeneity is an emerging theme in oncogenesis. In addition to the well-appreciated genetic and epigenetic heterogeneity of cancer cells, tumors also significantly differ in terms of quantity and phenotypic characteristics of immune and stromal cells that are being recruited, causing a high degree of cellular plasticity within the TME. First, this plasticity exhibits itself in various types of cell polarization, driven by distinct transcriptional programs. This enables cells with functions that are essential for tissue regeneration or for tumor development, but that are typically not found in these cells under unchallenged conditions. Second, spatiotemporal cellular plasticity allows the transition of cells from one state to another during such processes as epithelial-mesenchymal transition (EMT), cell migration, mesenchymal-epithelial transition (MET), and metastasis. It is likely that "subtypes" or "types" previously assigned to different cells within the TME as states of terminal differentiation or distinct cell types often represent differentiation and activation states, which might undergo further phenotypic and functional changes through the cell plasticity. Plasticity can be presented by different cell types during different stages of tumor development. For example, during initiation stage of intestinal cancers, stem cells are proposed to be an origin of cancer; however, mutated epithelial and transient-amplifying cells can undergo plasticity and dedifferentiation to acquire a cancer stem cell phenotype in response to NF-kB activating inflammatory signaling. However, the best-known case of cancer cell plasticity is through their ability to undergo various states of EMT and MET and re-establishment of cancer stem cell pool during metastasis or therapy. All of these processes are strongly influenced and regulated by inflammation, cytokines, and growth factors. However, it is not only cancer cells that are subject to plasticity. Essentially all other cell types in the TME are engaged into reciprocal activation/inhibition/differentiation signals to influence and drive plasticity. For example, cancer cells can utilize vesicular transport and exchange to shape plasticity of dendritic cells by supplying them with both antigens and tumor derived cues, ultimately to shape T cell responses. Cancer cells can also instruct fibroblasts to acquire differentiation states that are tumor-promoting and immunosuppressive, but activation of fibroblasts can also be driven by cytokines, such as IL-6 or IL-17, and released by immune cells. Cancer-associated fibroblasts (CAFs) activated by inflammatory signals might be either pro- or anti-tumorigenic, and depending on their proximity to cancer cells as well as their response to IL-1R engagement or TGF-b activation, they might differentiate into inflammatory CAFs (iCAFs) or myofibroblasts (myCAFs). CAFs in return can provide differentiation, growth factor, survival, and metabolic cues to cancer cells and maintain the cancer stem cell niche, thereby promoting growth, metastasis, and resistance to therapy. CAFs can also regulate myeloid and T cell infiltration and produce factors that regulate immune cell differentiation and plasticity and drive immunosuppressive phenotypes. On the other hand, under different circumstances, CAFs or inflammatory signaling in CAFs might also be tumor suppressive Myeloid cells also present with a high degree of plasticity regulated by the TME. Tumor cell derived factors (such as TGF-b), other cytokines, metabolic cues, or products of dead cells polarize local macrophages or incoming monocytes toward more "tissue repair" type, characterized by enhanced expression of tissue-protective factors, growth factors, and decreased expression of genes involved in antigen presentation and induction of antigen-dependent immune responses. Such macrophages were previously referred to as "M2 type macrophages"; however, by now it has become evident that, in the complex context of the TME, such a clear distinction does not exist and that *in vivo* macrophages can express genes of both "former M1" and "former M2" types. The instructing signals for macrophage polarization also can come from infiltrating bacteria in bacteria-rich cancers or from the cells of adaptive immunity such as B cells, T helper 2 (Th2) cells, or T regulatory cells. On the other hand, Th1 and CD8+ T cells, particularly via interferon-g (IFN-g)-dependent mechanisms, can drive polarization of tumor-associated macrophages (TAMs) toward the "tumor-ignorant" functional phenotype that would be devoid of secretion of growth factors and characterized by increased phagocytosis, antigen presentation, and cross-presentation, stimulating anti-tumor immune responses. Importantly, different tumor entities seem to educate their individual macrophages causing cancer-specific TAM profiles. Moreover, macrophage reprogramming may be induced by triggering their integrins and surface receptors, such as CD11b. Similarly to macrophages and monocytes, the TME also imprints a significant degree of plasticity on tumor-associated neutrophils via cytokine signaling or regulation of metabolism to further shape them to be anti-tumorigenic and antigen-presenting, directly tumor-promoting, or immunosuppressive. T cells and B cells represent the adaptive immunity arm in cancer and also possess a tremendous predisposition to plasticity within the TME. B cells recruited into tumors regulate anticancer

immune responses, exert direct tumor promoting roles, or modulate myeloid cell response, all of that dictated by the signals B cells receive from TME and by the nature of ligands their B cell receptors (BCRs) recognize. Gammadelta T cells are important components of tumor immunesurveillance, but in the TME, they can acquire tumor-promoting functions associated with cytokine expression or act as negative regulators for T cell activation in pancreatic cancer. Alfabeta T cells also possess a great degree of plasticity within the TME, which is being impinged in situ and in the secondary lymphoid organs where priming of these cells occurs. Signaling by cancer cells through the PD-L1-PD-1 pathway, inducible by inflammatory cytokine IFN-g, can render CD8+ and CD4+ T cells exhausted and similar processes can be enforced by regulatory T cells and by metabolic products of TME, such as hypoxia, glutamine, or potassium ions. Microbial and inflammatory cytokine signaling can drive normally immunosuppressive and anti inflammatory FoxP3+ Tregs to co-express transcription factor RORgt, creating a pro-inflammatory regulatory plastic T cell lineage capable of producing tumor-promoting cytokines IL-17 and IL-22, while being generally suppressive for anti-cancer immunity.[37,38]

INFLAMMATION AS AN ANTI-CANCER THERAPEUTIC OPPORTUNITY[21,22,24,32,34,39]

NON-SELECTIVE STRATEGIES

Harnessing and manipulation of the inflammatory response for therapeutic benefit is an area of intense interest. Broadly, strategies can be divided into those that target systemic inflammation more generally, often via unknown targets, and those that selectively alter specific pathways or components of the immune and inflammatory response within the local tumour microenvironment or systemic circulation. Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), including cyclo-oxygenase-2 inhibitors, have potential as therapeutic drugs in patients with cancer. In colorectal cancer, consistent evidence shows their chemopreventive action, with reduced prevalence and mortality shown in epidemiological studies.[15,27,28] NSAIDs have likewise shown the ability to alter systemic inflammation and improve moderate cachexia and the results of studies have likewise shown a benefit in early stage disease, with a reduction in tumour recurrence. Perioperative NSAID use has been shown to decrease recurrence and mortality in patients undergoing curative resection for cancer, examined in patients with a high preoperative NLR, and in patients with colorectal cancer, with improvements in both recurrence and mortality of as much as 40%, which gives rise to the idea of NSAID or aspirin use as adjuvant therapy for colorectal cancer, with several large-scale phase 3 clinical studies now underway. The mechanism of action of NSAIDs, including aspirin, is not fully understood and several pathways have been implicated in their direct and indirect effects on tumour cells, the tumour microenvironment and the innate and adaptive immune responses. Inhibition of several transduction pathways, including Wnt-β catenin; PIK3CA/AKT/PTEN; and NF-κB, has been postulated because patients with PIK3 mutations show increased survival compared with wild-type patients, and those with mutations within the NF-κB pathway could have reduced cancer risk after use of NSAIDs. There is good evidence for the effect of NSAIDs and aspirin on platelet function, with some indication that they might have a role in the enhancement of cytotoxic T-cell activity, with the potential prevention of cancer-associated immune suppression. The authors of several studies have examined the effect of aggressive systemic anti-inflammatory treatment on chemotherapy efficacy and its toxic effects.

High-dose dexamethasone-up to 16 mg twice a day before chemotherapy in clinical settings-has improved efficacy of chemotherapeutic drugs, decreased haematological toxic effects, and altered drug pharmacokinetics, all of which have been associated with significantly decreased drug uptake by host tissue. Statins have the potential for adjunctive anticancer treatment in view of their known effects on cell proliferation, inflammation, angiogenesis, and endothelial cell function. A reduced risk of colorectal cancer has been reported from epidemiological studies of concomitant statin use, with an up to 90% risk reduction in inflammatory bowel disease-related colorectal cancer.

Statins could likewise alter immune and inflammatory responses in cancer, on the basis of extrapolation from the results of cardiovascular disease prevention studies, in which pro-inflammatory cytokines were downregulated in patients with increased serum inflammatory markers. Studies in patients undergoing cardiac and renal transplantion suggest potential immunomodulatory effects, with evidence of decreased organ rejection, potentially through a direct effect on MHC class II expression and subsequent T-cell activation.

TARGETED STRATEGIES

In view of the overlap of cytokines and small inflammatory proteins present in both the local tumour micro environment and systemic circulation, drugs targeted to specific mediators will affect both the local immune response and

systemic inflammation. The tumour microenvironment and local immune response presents an ideal target for anti-cancer treatment since non-tumour cells are more likely to be genetically stable compared with tumour cells, which therefore eliminates the issue of acquired resistance to chemotherapeutic drugs. The delicate balance, however, between the tumour-inhibitory and tumour-promoting properties of the microenvironment, together with the normal physiological role of stromal cells, means that therapeutic approaches should selectively target their cancer promoting properties and leave their other activities intact. Although much information is available about selective targets for therapy and drugs under early-phase clinical investigation, there are few clinically active drugs. Ideal targets include microbial/viral/fungal infectives, cytokines, chemokines, growth factors, components of the extracellular matrix such as matrix metalloproteinases, enzymes of cysteine and cathepsins, and integrins. Given the extensive molecular intersections and crosstalk, cellular adaptability, and organ-specific contexture, anti-inflammatory therapy targeting a single immunomodulatory factor might lead to tumour evolution or TME remodelling. Together, the paradoxical findings discussed above should prompt special caution when translating anti-inflammatory therapies into clinical use. To analyze all the aspects and pros vs cons of anti-tumoral/anti-inflammatory therapeutic strategies is beyond the scope of this overview.[21,24,34,39] The numerous and diverse links between cancer and inflammation all present therapeutic opportunities, especially when the concept of 'inflammation' is broadened to include viral, bacterial and fungal infections. However, numerous hurdles and uncertainties remain in every step of translating an anti-inflammatory agent into clinical use. First, whether the inflammatory redundancies identified in preclinical models are targetable and druggable remains unclear. Second, the heterogeneity and plasticity of the TME present problems in targeting a single cytokine or even a single cell type. The effects of disrupted negative feedback loops and the activation of compensatory pathways is also hard to predict. Third, given that patients with cancer are typically assigned to conventional treatments, a need exists to identify more specific inflammatory targets that are responsible for therapy resistance or adverse events. Fourth, contrary to other targeted therapies, clinically applicable biomarkers for the selection of anti-inflammatory agents and assessment of their anticancer effects are lacking. Fifth, the effects of endogenous factors, such as the patients age and microbiota, on the magnitude of inflammatory responses and on the outcomes of anti-inflammatory treatments remain to be determined. Notably, the composition of the microbiota has been shown to affect immunotherapy efficacy, suggesting that microbial interventions could potentially be leveraged to improve cancer prevention or treatment. By necessity, defining a therapeutic paradigm for anti-inflammatory treatments would require optimized pharmaceutical programmes as well as appropriate animal models and clinical trial designs. In addition, integrative high-resolution analyses using multiomics, single-cell and/or spatial-based technologies should provide deeper insights into local therapeutic responses and the exact cellular and molecular consequences of anti-inflammatory treatments. Finally, the deployment of personalized, multi-agent, anti-inflammatory regimens in the era of immunotherapy is possibly another key to treating cancer.

CONCLUSION

Cancer is a research topic, a medical/surgical problem, and a social issue. Too many lives are lost each year to cancer. Many types of barriers still exist between patients in need and effective treatment/care. These go beyond knowledge and technical limitations raising many (de-)pressing issues. How can we overcome not only the difficulties/complexities of tumor biology itself but also the tumor microenvironment it resides? Research indicates that interactions between the neoplastic cells, the extracellular matrix, and stromal cells are bidirectional and dynamic. TME can exert both stimulatory and growth inhibitory effects on tumor cells. Cancer cells are often critically dependent on the stroma, as is the case between neoplastic and endothelial cells. The interplay between local and systemic inflammation and tumor promotion, progression, and metastasis is complex and includes components of the adaptive, humoral, and innate immune systems. Given that inflammation can play an instrumental role during all stages of tumor development, future research will undubtedly uncover molecular and cellular mechanisms and modes of operation for inflammation and immune cells. As the array of modern cancer therapies continously expands, including various therapies based directly on immune cells(checkpoint immunotherapies, vaccines, and CAR T cells), it will be imperative to uncover the role of immune and inflammatory pathways in therapy resistance.

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Trauma and Inflammation

Travma ve İnflamasyon

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ABSTRACT Management of severely injured patients is a complicated process with so many questions that need to be answered. Understanding the immune mechanisms and complex physiological responses to trauma is at utmost importance to provide appropriate and up-to-date care. The organism's immune response to trauma is a balanced interactions of inflammatory and anti-inflammatory mediators which purpose is to eliminate the insult faced by the organism and returning it to its pre-injury state. Initial inflammatory response after a trauma described on two main molecular patterns: Damage associated molecular pattern (DAMP) and pathogen associated molecular pattern (PAMP). These patterns act as "signal 0". DAMPs are well described inflammatory trigger mediators. In severely traumatized patients, treatment protocols should be made with considerations on described immune responses and their clinical effects. Staged approach with "*damage control*" philosophy should be treatment of choice according to current published literature.

Keywords: Inflammation; immune response; trauma

ÖZET Travma hastalarının yönetimi, cevaplanması gereken pek çok soruyu barındıran karmaşık bir süreçtir. Organizmanın travma sonrası verdiği immün yanıtı, aktive olan biyolojik yolakları ve ilişkilerini anlamak uygun travma bakımını sağlayabilmek için son derece önemlidir. Organizmanın travmaya karşı verdiği immün yanıtı; organizmanın karşılaştığı tehdidi ortadan kaldırmak ve onu yaralanma öncesi durumuna geri döndürmek amacı doğrultusunda, inflamatuar ve anti-inflamatuar mediyatörlerin dengeli ve kontrollü bir etkileşimi olarak özetlenebilir. Travmadan sonraki ilk inflamatuar yanıt, iki ana moleküler model üzerinden tanımlanır: Hasarla ilişkili moleküler patern (DAMP) ve Patojenle ilişkili moleküler patern (PAMP). Bu modeller "sinyal 0" (başlangıç noktası) olarak işlev görür. DAMP'ler iyi bilinen inflamatuar tetikleyicilerdir. Travma olgularında tedavi planlaması, tanımlanan immün yanıtlar ve bunların klinik etkileri dikkate alınarak yapılmalıdır. "Hasar kontrolü" felsefesi ile basamaklı yaklaşım, güncel yayınlanmış literatüre göre tercih edilmesi gereken tedavi yöntemi konumundadır.

Anahtar Kelimeler: İnflamasyon; immün yanıt; travma

Despite all advances throughout medical care and injury prevention strategies, trauma still holds the first place for cause of death in young population. Polytrauma on the other hand, is a complex interaction of different mechanisms. Improvements in early resuscitative care and appropriate triage led to a decrease in early mortality after trauma. But head trauma and massive hemorrhage are still most common causes of early mortality after trauma. Late mortality after trauma is mostly caused by "multiple organ failure (MOF)" and "acute respiratory distress syndrome (ARDS)".[1]

Mortality after trauma distributes trimodally (Figure 1). First peak represents immediate deaths at the injury scene and this phase can only be addressed by injury prevention. Fifty percent of traumatic deaths are in first peak. Second peak represents deaths related to trauma within 48 hours after injury caused by hypoxia, hypovolemia, and cerebral hemorrhage. Second peak in mortality can be addressed by well-established trauma

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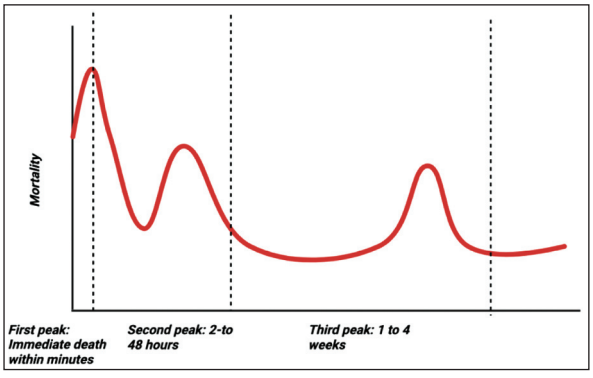


FIGURE 1: Trimodal distribution of traumatic deaths.

care system. Third peak of trauma related deaths occur within 1 to 4 weeks after trauma, and it represents deaths caused by complications of trauma or treatment related to trauma. SIRS and MODS being possible suspects of death, research of inflammatory mechanisms in trauma and concept of damage control surgery aims to reduce mortality in this period.[2]

Recovery pattern after severe traumatic event dictated by blood loss, change in tissue perfusion, immune system activation and coagulation system. Molecular mechanisms of inflammation after major injury still holds many questions that need to be addressed. Biological responses of the traumatized organism to the trauma basically have 2 main purposes: to survive and to restore pre-traumatic homeostasis. Traumatic injury leads to activation of immune system through both local and systemic reactions. Initial response to trauma results in proinflammatory mediator production.[3]

Traditionally, pathophysiological pathways after major trauma have studied under two main headlines: initial traumatic impact which called first hit and second hit that can be resulted from either excessive immunoinflammatory response or a surgical intervention. Briefly, inflammation is a physiological response to an insult to a living tissue. Purpose of inflammatory response is to eliminate the insulting agent, prevent further damage and to heal. Aim of this section is to describe inflammatory reactions to trauma and their role in maintaining homeostasis.

INITIAL RESPONSE TO TRAUMA

Traumatic insults disrupt macro and micro barriers, which ignites series of biological responses thru different pathways. Tissue destruction such as fracture, laceration or any other kind of soft tissue damage, acidosis, hypoxia, and pain activates local and systemic immune reactions. Some being depended to injury pattern and severity, some of the activated molecular pathways is freed from trauma's severity or type but after all, patient's age, sex, comorbidities, type of injury defines the magnitude of total immun response.[4,5]

Initial inflammatory response after a trauma described on two main molecular patterns: Damage Associated Molecular Pattern (DAMP) and Pathogen Associated Molecular Pattern (PAMP) (Table 1). These patterns act as "signal 0". DAMPs are well established inflammatory trigger. Both DAMPS and PAMPS could interact with neutrophils thru PRRs (Pattern Recognizing Receptors). Since the TLR 4 being most studied among them, they are considered as a target molecule in possible future anti-inflammatory interventions (Table 2).[6]

DAMPs released after tissue injury directly activates several immune cells such as monocytes and neutrophils via DAMP receptors. Altogether DAMPs recognized by immune system initiates a hyperinflammatory state (Table

TABLE 1: Molecular patterns in initial inflammatory response after trauma.

DAMP	PAMP
Burns	Bacterial infections
Trauma	Fungal infections
Surgical Intervention	Toxic Shock Syndrome from exotoxins/endotoxins
Ischemia	*External (non-self) factors*
Released by injured host cells *(self-induced)*	Recognized by Pattern Recognition Receptor (PRR) bearing innate immune system cells
Binds to its specific receptors to promote inflammation and initiate tissue repair	Most of them are lipopolysaccharide
Transmit their signals thru TLRs, NLRs RAGE, purinergic receptors and complement receptors	Transmit their signals thru TLRs, NLRs, RLRs RAGE, purinergic receptors and complement receptors
Potent complement activator	

TABLE 2: Described DAMPs and their receptors.[8]

References	DAMP	Damp Receptor
Cytosol	Uric Acid	NLRP3, P2X7
	S100 Proteins	TLR2, TLR4, RAGE
	HSP	TLR2, TLR4, CD91
	ATP	P2X7, P2Y2
Nucleus	Histones	TLR2, TLR4
	HMGB1	TLR2, TLR4, RAGE
	HMGN1	TLR4
Mitochondria	TFAM	RAGE
	mROS	NLRP3
Plasma Membrane	Syndecans	TLR4
	Glypicans	TLR4
Extracellular Matrix	Biglycan	TLR2, TLR4, NLRP3
	Decorin	TLR2, TLR4
	Fibrinogen	TLR4
	Tenascin C	TLR4

2). Clinical reflection of this hyperinflammatory state could be hypo/hyperthermia, elevated hearth rate, and leukocytosis/leukocytopenia. DAMPs also activate complement system resulting in elevated C3a and C5a serum levels. Consumption and imbalance in complement cascade components after prolonged inflammatory response could impair immune system further.[7]

Physiological response to any kind of insult, is balanced and controlled combination of humoral and cellular immune responses. Both proinflammatory and anti-inflammatory pathways are activated with initial trauma and balance between them is maintained throughout the healing process. Excessive or uncountered proinflammatory activation could lead to a Systemic Inflammatory Response Syndrome (SIRS). While SIRS is well-known and established term, there is other homeostatic mechanism called Counter Anti-inflammatory Response Syndrome (CARS) which initiates simultaneously. Balanced response would clear the molecular danger and induce tissue healing mechanisms (Figure 2).

SIRS is countered with CARS. CARS is defined by elevated levels of anti-inflammatory cytokines (Table 3). With the aid of balance between pro-inflammatory and anti-inflammatory system, immun response might be adequate and return the homeostasis to baseline or can progress to persistent inflammation resulting with multiorgan dysfunction/sepsis. CARS is caused by suppressing DAMPs (SAMPs).[9]

Activation of leukocytes, monocytes, lymphocytes, and natural killer cells leads to a key factor in systemic inflammatory response: endothelial membrane dysfunction. Polymorphonuclear leukocytes interact with endothelial membrane thru adhesion molecules. Activated neutrophils migrate thru blood stream and endothelial damage occurs

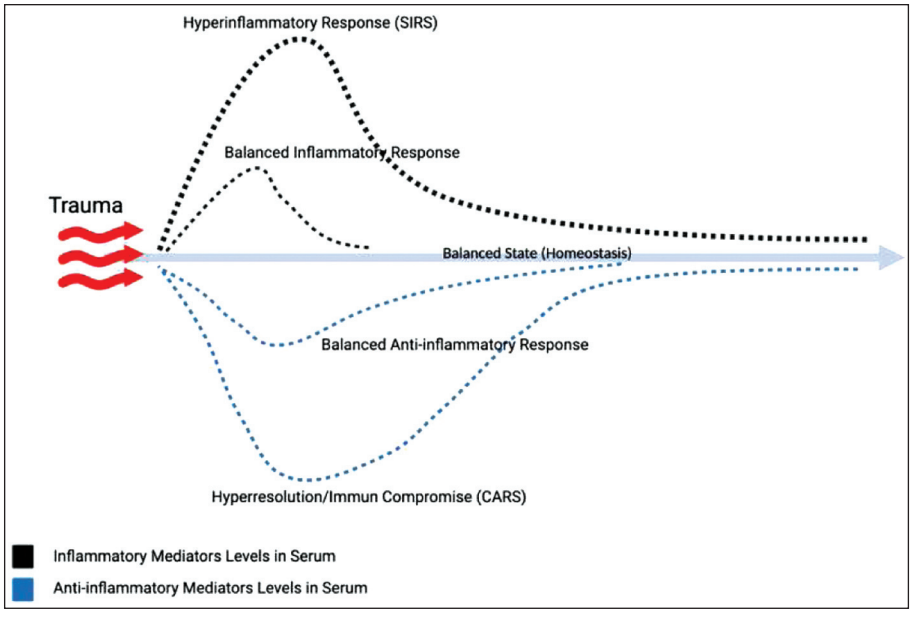


FIGURE 2: Serum levels of inflammatory and anti-inflammatory mediators.

TABLE 3: Anti- and pro-inflammatory mediators elevated in serum.

Anti-inflammatory Mediators	Pro-inflammatory Mediators	
IL-10	IL-1 β	IFN-γ
TGF-ß	IL-4	HMGB-1
IL1-Ra	IL-6	MPO
	TNF-α	Elastase

not only at the injury site, also at vital organs such as lungs and kidneys. Endothelial damage marks the endpoint for initial immune response after trauma. Extensive initial trauma or extensive damage by any other mean (surgical intervention, hypovolemic shock etc.) could imbalance the initial immune response that could result in:

- Dysregulated cascade system activation which can result in trauma induced complementopathy or coagulopathy.
- Excessive inflammatory response resulted in cytokine storm that leads to cell membrane dysfunction

SYSTEMIC RESPONSE TO TRAUMA

Endothelial dysfunction after aforementioned mechanisms implicates pathologic changes throughout the organism. Increased permeability at endothelial barrier manifests as loss of intravascular volume. Intravascular volume shifts to *third space.* Loss of intravascular volume leads to hypoperfusion. Effects of hypoperfusion in organs summarized in (Table 4).[10]

SIRS (Systemic Inflammatory Response Syndrome): SIRS defines organisms' excessive response to an insult. Trauma being one of them, several agents such as infec-

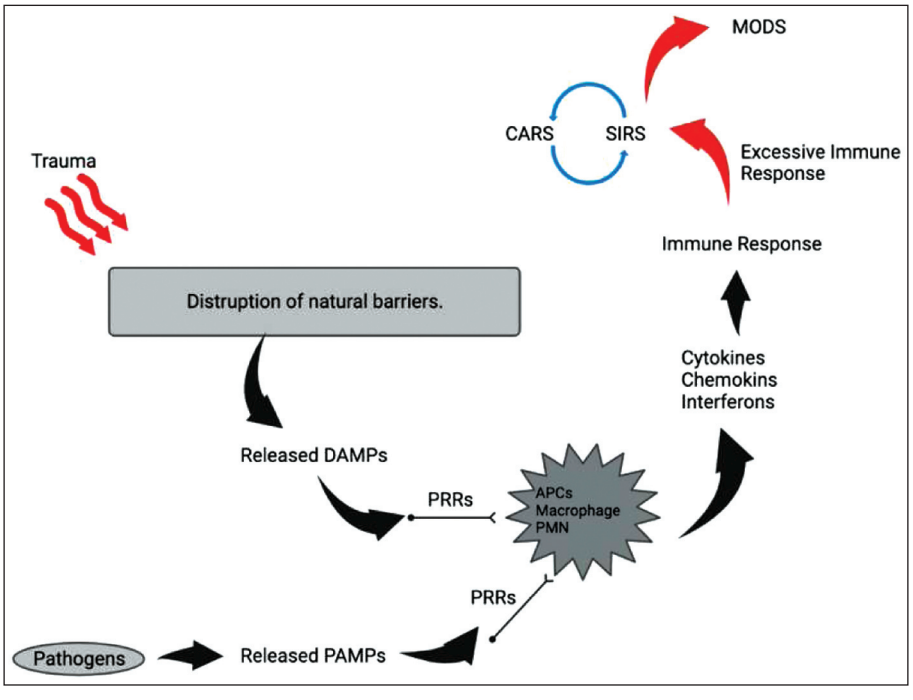


FIGURE 3: Immune response to trauma.

TABLE 4: Effects of hypoperfusion on vital organs after systemic immun response to trauma.

Organ	Effect	Complication
Kidney	Lowered Urinary Output	Acute Renal Failure
Lung	Hypoxemia	ARDS
Brain	Impaired perfusion	Altered mental status
Intestines	Translocation of pathogens (and related PAMPS) into body	Sepsis

tion, ischemia, burn, malignancy etc. could cause systemic inflammatory response syndrome (Table 5). Even though inflammatory response to a stress factor aims to heal and control the damage, excessive stimuli can provoke systemic response. Excessive and dysregulated immune response acts thru endothelial dysfunction and release of proinflammatory cytokines to bloodstream. Elevated mediators end up creating cytokine storm and persistent activation of different immune mechanisms that may lead to an irreversible end-organ damage, even death. Core mechanism for SIRS is increased permeability in endothelium that starts locally then involves multiple organs.[11]

Multiple Organ Disfunction Syndrome (MODS): This term defines a status initiated by trauma, shock or infection and led to a dysfunction of organs (two or more). MODS can be considered as a late excessive immune response to an insult to organism. Systemic inflammatory markers and elevated mediators in serum is similar to SIRS, but in MODS there is need for supportive interventions to maintain homeostasis (dialysis, ventilation support, etc.). In later stages of systemic response to a traumatic event, sepsis can occur. While definition and criteria for sepsis is still on debate, briefly it can be explained as additional immune compromise caused by infection.

TABLE 5: SIRS criteria, two or more of the following meets the criteria.

SIRS Criteria
Body Temperature >38° or <36°
Hearth Rate >90 BPM
Respiratory Rate >20 per minute or PCO_2 <32
Leukocyte Count >12.000/μL or <4000/μL

MANAGING PATIENTS WITH TRAUMA

Managing patients with severe trauma, the timing and the extent of any treatment or intervention should be considered to avoid or minimize "second hit damage". Definitive treatment or reconstruction procedures should be delayed on behalf of minimizing risking secondary insults to patients after major trauma. Managing polytrauma/severe trauma patient, the first step should be triaging the injuries. Injuries that require immediate intervention should be addressed rapidly and treated accordingly. There are several guidelines and scoring systems for initial assessment and decision making, but common conclusion to all of them is to resuscitate and manage the life-threatening injuries first and wait for the window of opportunity for secondary definitive procedures to avoid a second hit.

Staged approach could be implied in those patients:

- Acute phase: Resuscitation, life-saving interventions.
- Primary Stabilization Period: *Damage Control Surgery (DCS).* Major extremity injuries, compartment syndrome and arterial injuries
- Secondary Period: Reassessment, avoid second hit
- Window of Opportunity: 5-10 days. Definitive treatment.

PRINCIPLES OF DAMAGE CONTROL SURGERY

The term "Damage Control Surgery" addresses a philosophy of keeping patients alive rather than rush to treat its non-lethal injuries. This approach described in multitrauma patients and its rationale is based on serum levels of pro and anti-inflammatory mediators in serum. Damage control philosophy can be studied under 5 phases (Figure 4).

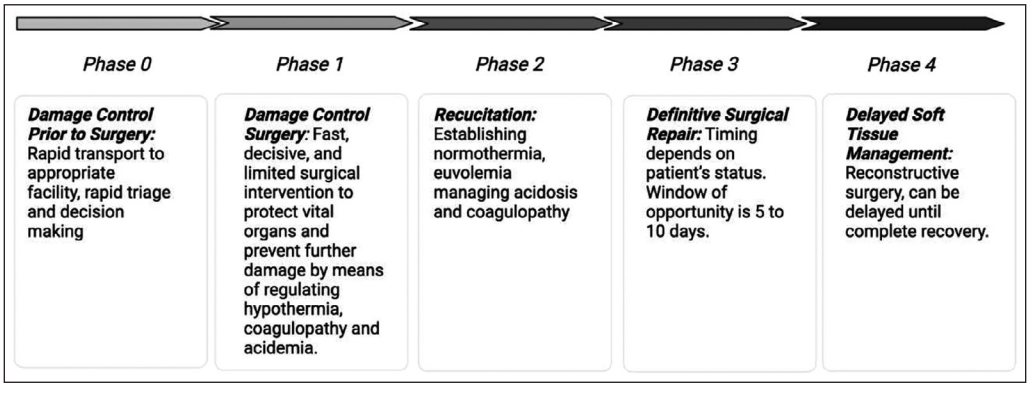


FIGURE 4: Phases of damage control philosophy.

Historically, early total care was primary principle applied in severely traumatized patients. With advances in implant technology and understanding more of immun responses after trauma, early total care philosophy is replaced with damage control surgery. While early fixation of unstable long bone fractures showed to be reducing mortality rates, it should be done without long-definitive operations that could make a second hit impact on inflammatory responses. At this point physicians could use external-fixators and skeletal tractions for temporary stabilization.[12]

Although damage control philosophy brought a new aspect to trauma care and reduced mortality rates in severely traumatized patients, most trauma patients still can be safely treated with early total care procedures. This brings a debate on cost-effectiveness and complication rates on damage control surgery. Although there are several studies on describing objective parameters in decision making on surgical intervention timing, more data with more studies needed to embed this approach.[13,14]

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Gut and Inflammation

Bağırsak ve İnflamasyon

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ABSTRACT Proinflammatory and anti-inflammatory cytokines produced by various cells of the mucosal immune system in response to various environmental factors, primarily to intestinal microflora, play a fundamental role in the pathogenesis of IBD. Numerous genetic factors that regulate epithelial function or inflammatory response pathways interact with each other to determine mechanism and the extent of response to be generated in the cells of the mucosal immune system against these external factors. Dendritic cells, neutrophils, macrophages, NK cells, intestinal epithelial cells, and innate and adaptive lymphoid cells (Th1, Th2, Th17), which are the components of the mucosal immune system acting under the influence of environmental and genetic factors, produce cytokines that cause mucosal inflammation. As a result, the balance between proinflammatory and anti-inflammatory cytokines is disrupted, leading to inflammation, development of inflammatory bowel disease (IBD), and persistence of the mucosal inflammation. The understanding of the pathogenesis of IBD has come a long way in recent years. The mechanisms associated with IBD discovered till date and those to be discovered in the future offer therapeutic promise.

Keywords: Inflammation; inflammatory bowel disease; celiac disease; microscopic colitis

ÖZET Bağırsak mikroflorası başta olmak üzere çeşitli çevresel faktörlere cevap olarak mukozal immun sistemin çeşitli hücreleri tarafından üretilen pro-inflamatuar ve anti-inflamatuar sitokinler inflamatuar bağırsak hastalığı (İBH) patogenezinde temel rol üstlenmektedir. Epitelyal fonksiyonu veya inflamatuar cevabı düzenleyen yolaklarla ilgili düzenleyici çok sayıda genetik faktör birbiriyle etkileşim halinde, mukozal immun sistem hücrelerinin bu dış etkenlere karşı nasıl ve ne şiddette yanıt oluşturacağını belirler. Çevresel ve genetik faktörlerin etkisi altındaki mukozal immun sistemin bileşenleri olan dentritik hücreler, nötrofiller, makrofajlar, NK hücreleri, intestinal epitelyal hücreler, doğal ve adaptif lenfoid hücreler (Th1, Th2, Th 17) mukozal inflamasyona yol açan sitokinleri üretirler. Sonuçta pro-inflamatuar ve anti-inflamatuar sitokinler arasındaki dengenin inflamasyon lehine bozulması İBH gelişimine ve mukozal inflamasyonun devam etmesine neden olur. İBH'nin patogenezinin anlaşılması yolunda geçtiğimiz yıllarda gerçekten önemli mesafeler kat edilmiştir. Bugüne kadar bulunan ve bundan sonra keşfedilecek İBH ile ilişkili mekanizmalar, tedaviye yönelik umutlar yaratmaktadır.

Anahtar Kelimeler: İnflamasyon; inflamatuar bağırsak hastalığı; çölyak hastalığı; mikroskopik kolit

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a chronic disease with relapses and remissions that develop due to inflammation in the gastrointestinal tract (GIT).[1] IBD is classified as ulcerative colitis (UC), Crohn's disease (CD), and indeterminate colitis, which are the cases that do not fit into these groups. UC is a recurrent inflammatory disease and is usually limited to the colon without transmural involvement. Inflammation of the ileum and backwash ileitis may also be observed in a small number of patients. CD is a recurrent IBD affecting the entire GIT with transmural involvement of the mucosa.[2]

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ETIOLOGY AND PATHOGENESIS

The pathophysiology of the disease has not yet been fully elucidated. The pathogenesis of IBD is thought to involve an inappropriate and sustained inflammatory response against the common gut microbiota in a genetically predisposed host under the influence of environmental and microbial factors.[3]

GENETIC FACTORS

The biggest risk factor of IBD is the having a family member affected by IBD.[4] In patients having first-degree relatives with IBD, the risk of developing IBD is up to five times higher than in the healthy population.[5] The incidence rate of a positive family history for CD is higher than that of UC.[4] Patients with a family history of IBD are diagnosed with IBD at a younger age than those without a family history of IBD.[6] Owing to technological advances in genetic testing and analysis, many genome-wide association studies (GWAS) have identified single nucleotide polymorphism (SNP). Meta-analysis conducted by combining GWAS data identified 201 loci associated with IBD, including 41 and 30 loci specific to CD and UC, respectively. Approximately 68% of the identified loci were observed in both diseases, suggesting that UC and CD share common inflammatory processes.[7]

In 2001, the nucleotide-binding oligomerization domain-containing 2 (NOD2) gene on chromosome 16 was identified as a susceptibility gene for CD.[8] The NOD2 gene, a member of the NOD-like receptor family, encodes a protein that acts as an intracellular receptor for muramyl dipeptide (MDP) present in both gram-negative and gram-positive bacteria.[9] Stimulation of MDP in turn stimulates autophagy, which controls bacterial proliferation and antigen presentation, and regulates innate and adaptive immune responses.[10-12] NOD2 also functions in pathways such as the T cell response pathway, which is unrelated to MDP.[13] It is accepted by many researchers that malfunctioning of NOD2 leads to impaired bacterial clearance and increased inflammation; therefore, loss of NOD2 functionality is an important factor in the onset of CD.[14] The association between the ATG16L1 and IRGM genes, which are involved in autophagy, and CD has been identified using GWAS.[15,16] Gene products from autophagy are involved in innate and adaptive immune system reactions, responding to microorganisms, and their functioning; and disorders in this mechanism can cause the pathogenesis of CD.[17] The widespread use of genetic studies revealed the association between the IL23R gene and IBD.[18] The IL23R gene encodes a subunit of the interleukin-23 (IL-23) receptor and is involved in the development of Th17 cells via proinflammatory action of the cytokine. The Th17/IL-23 pathway, which involves IL23R, IL12B, JAK2, and STAT3 genetic susceptibility loci function, has been reported to be involved in the pathogenesis of IBD since it has been identified in the pathogenesis of both UC and CD.[19]

MICROBIOTA

GIT microbiota has many important effects on human physiology and metabolism. Synthesizing vitamins, regulating intestinal secretion and motility, removing difficult-to-digest nutrients from food, and regulating mucosal tolerance by modulating the immune system in the gut are among the main functions of the microbiota in the GIT.[20] The gut microbiota is established in the first two weeks of life and remains the same thereafter. Although only 20%–30% gut microbiota has been isolated, changes in the microbiota have been found to be associated with IBD. Several studies that examined the intestinal flora of both inflamed and noninflamed intestinal segments of patients with UC and CD revealed lower biodiversity of fecal microbiota in patients with UC and CD than the healthy individuals.[21] The predominant microbial species in the healthy gut belong to the phyla Firmicutes and Bacteroidetes, which support the production of epithelial metabolic substrate. Patients with CD have low Firmicutes and Bacteroidetes and high enterobacteria cells in their intestinal microflora, while those with UC had low number of *Clostridium* species and overgrowth of *Escherichia coli* (*E. coli*) in their intestinal microflora.[22,23] Mucosa-associated *E. coli* is thought to play a role in the pathogenesis of CD as it is present in high number in in the ileum and colon of patients with CD.[24]

IMMUNE SYSTEM

Evidence from studies on IBD suggests that dysfunctions in the innate and adaptive immune system lead to the generation of abnormal intestinal inflammatory response in patients with IBD. Recent studies have suggested that intestinal inflammation in UC and CD cases occurs in different ways. Inflammation in CD is thought to be caused by a long-standing Th1 response, whereas inflammation in UC is associated with an unconventional Th2 response.[25,26] The newly identified Th17 cells have also been found to be involved in the development of inflammation in IBD.[27]

INNATE IMMUNE RESPONSE

The innate immune response is nonspecifically elicited within minutes or hours against the antigen by various cells

such as epithelial cells, neutrophils, dendritic cells, monocytes, macrophages, etc. The innate immune response begins with the recognition of microbial antigens through receptors such as Toll-like receptors (TLRs) on the cell surface and NOD-like receptors in the cytoplasm. Recent studies on IBD show that the behavior of cells involved in the innate immune response and the expression and function of NOD and TLR proteins are markedly different in individuals with IBD.[28]

Autophagy plays a major role in maintaining homeostasis within the cell and host defense against microorganisms.[28] CD has been associated with the ATG16L1 and IRGM genes that are involved in autophagy. Mutations of these genes can cause disruptions in the autophagy process, leading to an increased risk of CD. Disruption of the unfolded protein response caused by endoplasmic reticulum stress is involved in the pathogenesis of IBD by inducing apoptotic cell death.[29]

Impaired epithelial barrier and increased intestinal permeability in patients may play a role in the pathogenesis of IBD.[30] The first line of defense in the intestine is formed in the mucus layer, and many studies have been conducted on the contribution of mucus in preventing bacterial translocation and inflammation.[31] The second line of defense, i.e., the layer of specialized epithelial cells in the intestine formed by cells such as enterocytes, goblet cells, and paneth cells, functions as a barrier that restricts the passage of bacteria and helps in the release of antimicrobial peptides from the epithelial cells, which can explain the aberrant expression of antimicrobial peptide in patients with CD.[29,32]

ADAPTIVE IMMUNE RESPONSE

Adaptive immunity onsets within a few days, is related to the type and number of T cell, and is highly specific to the antigen. Th1 cells stimulate IL-12 and secrete high amounts of interferon IFN-γ, IL-12, and tumor necrosis factor TNF-α. Th2 cells secrete IL-4, IL-5, and IL-13.[33] Patients with CD produce higher levels of IFN-γ and IL-2 in their mucosal T cells than patients with UC and control T cells. Atypical natural killer (NK) cells in patients with UC secrete more IL-13 than in patients with CD and control T cells.[34] These results supported the correlation between CD and Th1 immune response and UC and Th2-mediated immune response.[35] Th17 cells are defined as a subgroup of T cells that produce high amounts of IL-17A, IL-17F, IL-21, and IL-22.[29] The transcript levels of IL-17A were significantly higher in intestinal mucosa of patients with IBD than healthy individuals.[36] Although there is insufficient information on the effect of regulatory T cells (Treg) in IBD, the number and function of $CD4^+$, $CD25^+$, $FoxP3^+$, and $CD8^+$ Treg cells may be affected in IBD that may contribute to the continuation of inflammation.[37] α4β7 integrin expressed by lymphocytes in lymphoid tissue of the GIT and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the intestinal venule endothelium are also the key mediators in gut-mediated immunity against IBD.[38]

In conclusion, proinflammatory and anti-inflammatory cytokines produced by various cells of the mucosal immune system in response to various environmental factors, primarily to intestinal microflora, play a fundamental role in the pathogenesis of IBD. Numerous genetic factors that regulate epithelial function or inflammatory response pathways (autophagy, innate immunity, and adaptive immunity) interact with each other to determine mechanism and the extent of response to be generated in the cells of the mucosal immune system against these external factors. Dendritic cells, neutrophils, macrophages, NK cells, intestinal epithelial cells, and innate and adaptive lymphoid cells (Th1, Th2, Th17), which are the components of the mucosal immune system acting under the influence of environmental and genetic factors, produce cytokines that cause mucosal inflammation. As a result, the balance between proinflammatory and anti-inflammatory cytokines is disrupted, leading to inflammation, development of IBD, and persistence of the mucosal inflammation. The understanding of the pathogenesis of IBD has come a long way in recent years. The mechanisms associated with IBD discovered till date and those to be discovered in the future offer therapeutic promise.

MICROSCOPIC COLITIS

Microscopic colitis (MC) is a disease mostly seen in the geriatric population and characterized by chronic or intermittent watery diarrhea that may lead to electrolyte imbalance and severe dehydration. There are two subtypes of MC: lymphocytic colitis and collagenous colitis. Although these two subtypes are clinically and epidemiologically similar, the main histological difference is the presence of thickened subepithelial collagen band (>7 μm) in collagenous colitis. Acute and chronic inflammatory cells can be seen in the lamina propria in both diseases, and the pathologic diagnosis of lymphocytic colitis can be made if at least 20 intraepithelial lymphocyte cells are observed for every 100 surface epithelial cells.[39]

Autoimmune diseases such as diabetes, thyroid dysfunction, psoriasis, and rheumatoid arthritis have been frequently reported in association with MC.[40-43] However,

association of MC with celiac disease is the most striking. It has been reported that changes compatible with MC may be observed in the colons of up to one-third of the total patients with celiac disease.[44,45]

EPIDEMIOLOGY

MC is generally observed in the elderly population; the mean age at the time of diagnosis is 50-70 years.[40-42] In some publications, it is reported to be more common in women than men.[46,47]

PATHOPHYSIOLOGY

The pathophysiological mechanism underlying MC is not fully understood yet despite the several proposed mechanisms, ranging from autoimmunity to immune dysregulation against luminal antigens such as various drugs and infectious agents. A study suggested that there was a remarkable decrease in *Akkermansia* species in patients diagnosed with MC than the healthy individuals.[48] This bacterium thickens the colonic mucin layer; and decrease in *Akkermansia* population leads to thinning of the protective mucin layer and exposes the colonic epithelium to the harmful components of the feces.[48] Another study suggests that the presence of *Helicobacter pylori* and MC are inversely associated, but the protective effect elicited by *H. pylori* is still unknown.[49] Autoimmunity is another pathophysiologically contributing factor. One of the studies proved its association with other autoimmune diseases such as diabetes, thyroid dysfunction, psoriasis, and rheumatoid arthritis. Another study showed that antinuclear, antigliadin, and anti-*Saccharomyces cerevisiae* antibodies were found at higher levels in patients diagnosed with MC than in the healthy control group.[50] Since the HLA-DR3-DQ2 allele, which is also observed in celiac disease, was also found in patients with MC, it could be inferred that there may be a similarity between the pathogenesis of the two diseases.[51-53] In addition, TNF2 allele, which can also be detected in celiac disease, was also found in patients with MC in some studies.[51-53] However, these autoimmune and genetic markers are not of diagnostic value in MC. A previous study reported that five out of six patients diagnosed with CC had food allergy and the symptoms alleviated upon discontinuing the consumption of the allergic food.[54] Many researchers have investigated that malabsorption of bile acid may be involved in the pathogenesis of MC.[39] In a Spanish study, bile acid malabsorption was found in 43% patients with MC and 86% patients responded to cholestyramine treatment.[55] However, few studies do not support this finding.[56,57] In some patients diagnosed with MC, serologic or stool culture findings of *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Clostridium difficile* infections have been found, indicating that these infections were the triggers for MC;[58-60] however, there is no clear evidence that these bacteria can cause MC. There is evidence that inflammatory mediators are increased in the presence of MC. It has been shown that the levels of metalloproteinases; growth factors such as transforming growth factor β, vascular endothelial growth factor, cytokines, nitric oxide, and prostaglandins; and inducible nitric oxide synthase (iNOS) elevates in colonic epithelial cells, resulting in increased nitric oxide production.[61,62] In addition, another study showed that the levels of nuclear factor κB, which is activated in colonic epithelial cells and is a transcriptional activator of iNOS, also elevates.[63] Budesonide was shown to decrease iNOS mRNA levels and this was considered as its probable mode of action.[64] Drugs have been extensively studied in the etiopathogenesis of MC. Among the drugs that have been suggested to cause MC, acarbose, aspirin, nonsteroidal anti-inflammatory drugs, proton pump inhibitors, H2 receptor antagonists, SSRIs, and ticlopidine have the highest probability to cause MC; whereas carbamazepine, flutamide, lisinopril, levodopa/benserazide, and statins can cause MC with moderate probability.[39] Abnormal collagen metabolism is another pathogenetic mechanism responsible for causing MC. Some studies suggest that the abnormal collagen metabolism is a response to chronic inflammation, whereas other reports indicate that it is related to a primary abnormality in the collagen synthesis. Transforming growth factor beta, vascular endothelial growth factor, and fibroblast growth factor are important mediators that are upregulated in collagenous colitis.[65-68] Budesonide treatment decreases levels of vascular endothelial growth factor.[66] In collagenous colitis, pericryptal fibroblasts can be activated and have high synthesis activity.[69] However, another study found no evidence of increased collagen synthesis, which was determined by measuring mRNA levels.[70] Since the severity of diarrhea in collagenous colitis is correlated with the severity of inflammation rather than the thickness of the collagen band, the opinion of increased activity of pericryptal fibroblasts as a response to inflammation seems reasonable.[71] In conclusion, although the underlying mechanism has not been fully elucidated, it seems undeniable that inflammation plays a role in the pathogenesis of MC.

CELIAC DISEASE

Celiac Disease (CD) is an autoimmune disease that mainly affects the small intestine in genetically susceptible individuals due to the consumption of gluten. For the onset of CD, a complex immune response including innate and adaptive immunity against gluten proteins is required.[72]

PATHOGENESIS

Although many features of CD have been described in many studies, the etiology and pathophysiology of the disease are not fully understood. Nevertheless, it is thought to be caused by a combination of genetic, environmental, and immunologic factors.

GENETIC FACTORS

Human leukocyte antigen (HLA)-DQ alleles encoding MHC-DQ2 and -DQ8 heterodimers comprise the necessary but insufficient immunogenetic infrastructure for the onset of CD. Although HLA-DQ2 and HLA-DQ8 heterodimers are important risk factors for CD, they are accountable for only 40% of the genetical mechanism of the disease.[73] Non-HLA genes are thought to account for 60% genetic predisposition to CD.

Intestinal permeability is another factor that might play a role in CD. Increased passage of incompletely digested gluten particles from the intestinal lumen to the lamina propria, i.e., leaky gut, may cause activation of the innate immune system in its early phases.[74]

THE ROLE OF GLUTEN

Gluten is a complex protein found in grains such as wheat, barley, and rye. It has higher proline and glutamine and lower charged amino acids than other grains. It comprises a combination of proteins called gliadins, which are harmful to people with CD. In individuals with CD, gluten proteins activate the adaptive immune system. The inability of gastric and pancreatic enzymes to degrade these proteins is a key factor in activation of immunogenicity. As gluten peptides are resistant to proteolysis by gastrointestinal enzymes, long peptides remain in the intestinal lumen. While gliadin peptides remain in the lumen, some peptides can cross the intestinal epithelial barrier and interact with antigen-presenting cells in the lamina propria, thereby causing either intestinal inflammation or increased permeability.

THE ROLE OF TISSUE TRANSGLUTAMINASE

Tissue transglutaminase (tTG) is a calcium-dependent enzyme that catalyzes post-translational protein modification and is released from the cells during inflammation. It is thought that tTG has at least two roles in CD: 1) a deamidating enzyme that potentiates the immunostimulatory effect of gluten and 2) a target self-antigen in the immune response.[75] This enzyme can be present in the extracellular matrix; in the cell interior, mostly in the cytoplasm; in the nucleus; and on the cell surface. It is usually stored in the cell in an inactive state. This enzyme catalyzes the covalent and irreversible crosslinking between glutamine-containing protein (glutamine donor) and lysine-containing protein (glutamine acceptor). In the absence of a suitable glutamine acceptor or in the presence of a low level of pH in intestinal inflammation, tTG deamidates only glutamine present in the substrate protein. tTG may itself aggravate inflammation in CD by damaging the extracellular villous matrix, and it is also associated with the humoral response that contributes to the progression of the disease.[72]

PATHOPHYSIOLOGY OF THE CELIAC DISEASE

Consumed gluten is partially digested by digestive enzymes of the host and enzymes of the microbiota present in the small intestine. This incompletely digested gluten usually passes transcellularly or paracellularly into the lamina propria. tTG is activated to deamidate glutamine residues in the gluten peptides, which enhances the peptide-binding activity of antigen-presenting cells via HLA-DQ2 or HLA-DQ8. tTG also mediates the crosslinking of tTG-gluten complexes that activate antibody-forming B cells. Gluten-specific CD4 T helper 1 cells secrete proinflammatory mediators such as IF-γ, IL-12, and tumor necrosis factor. The interaction of extracellular tTG and gluten peptides induces the formation of tTG-specific B cell clones that secrete autoantibodies against tTG. These antibodies are sensitive and specific markers for the serologic diagnosis of CD. B cells also internalize tTG-gluten complexes, release deamidated gluten peptides that bind to HLA-DQ2 or HLA-DQ8, and cause further activation of gluten-specific T cells. Infectious or inflammatory markers upregulate stress markers such as MHC class 1 chain-related proteins A (MICA) and B (MICB), HLA class 1 histocompatibility antigen, and alpha chain E (HLA-E) in intestinal epithelial cells. Cytotoxic transformation of intraepithelial lymphocytes interacts with intestinal epithelial cells via NK cell receptors. Proinflammatory cytokines such as IL-15 induce cytotoxicity of intraepithelial lymphocytes, leading to cell destruction and intestinal atrophy. Overexpression of IL-15 in the intestinal epithelium and lamina propria causes dysregulation of the immune system and epithelial damage.[72] New CD4 T subpopulations such as $CD4^+$ $CD25^+$ regulatory T cells and Th17 cells have been shown to play a role in inflammation. Both Treg and Th17 cells levels were elevated in the presence of active CD.[76] Gliadin-specific Th17 cells have been found in the mucosa of CD. These cells are thought to have a dual role in the pathogenesis of the disease. These cells secrete proinflammatory cytokines such as IL-17, IFN-γ, IL-21; protective mucosal IL-22; and modulator TGF-β. These cells can also regulate the production of IL-17A.[77] In the pathogenesis of CD, IL-33 and its fragments may aggravate

the proinflammatory environment and potentiate the cytotoxic activity of $CD8^+$ T cells. IL-21 plays an important role in the regulation of Th17 cell response and is overproduced in patients with CD.[78] Zonulin is a paracrine protein that regulates the opening of tight junctions in the intestine. It is released from many cells, including intestinal epithelial cells. Exposure to gliadin or imbalance in the microbiome (dysbiosis or proximal intestinal colonization) may stimulate the release of zonulin.[79] Gliadin interacts with the chemokine receptor CXCR3 on the intestinal epithelial cells and the monocytes, leading to MyD88-dependent zonulin release and increased intestinal permeability.[80]

MICROBIOTA

The oral microbiota is the largest microbial community in the body after the colon. In resistant CD cases, an increase in actinomycete, a decrease in actinomyces immunoglobulins, and a decrease in *Bacteroides* and *Fusobacteria* have been reported in oral microbiota.[81] On the other hand, *Bifidobacterium bifidum* was found to be higher in fecal microbiota of patients with untreated CD than in the healthy individuals. In treated patients with CD, the diversity of *Lactobacillus* and *Bifidobacterium* species decreases. The same study compared the control group of healthy individuals and patients with CD and revealed higher amounts of short-chain fatty acids such as acetic acid, propionic acid, and butyric acid in the feces of all patients with treated or untreated CD. Few patients with treated celiac disease were found to be able to restore their microbiota when compared to the healthy control group.[82]

In conclusion, CD seems to occur on the basis of a genetic predisposition and a permeable gut hypothesis, whereas it also develops an inflammatory response that the host generates due to its microbiota and the environmental factors. However, it seems that many areas of the etiopathogenesis of the disease still need to be elucidated.

DIVERTICULAR DISEASE

Diverticula are sac-like protrusions that can be seen along the entire GIT, most commonly seen in the colon. Diverticular disease (DD) has different subtypes: diverticulosis, which is characterized by the presence of asymptomatic diverticula; diverticular disease, which is a symptomatic uncomplicated disease with symptoms such as abdominal pain, changes in bowel habits, and is not accompanied by significant inflammation; and diverticulitis, which is characterized by inflammation of the diverticula. Diverticulitis can be also divided into uncomplicated disease with localized inflammation and complicated disease with fistula, intestinal obstruction, perforation, bleeding, and abscesses.[83] Despite the identification of risk factors for DD such as genetic dispositions, changes in diet and lifestyle, and prescribed medications, the impact of these risk factors on inflammation is yet to be understood.

Mucosal inflammation caused by bacterial overgrowth is thought to be the underlying mechanism of diverticulitis. However, the role of the immune system and mucosal inflammatory mediators in DD is not fully understood. The underlying mechanism of diverticulosis is thought to be neuromuscular abnormality and increased luminal pressure, with inflammation playing almost no role.[83] In diverticulosis, a single nucleotide polymorphism (SNP, rs3134646) in the alpha 1 chain of type 3 collagen has been shown to contribute to the formation of the disease by altering the collagen vascular system.[84] In biopsy samples collected from the colonic mucosa of patients with asymptomatic diverticulosis, no change was found in $CD4^+$, $CD8^+$ and $CD27^+$ cells and IL-6, IL-10, and TNF-α levels.[85]

On the other hand, low levels of inflammation and visceral hypersensitivity are thought to be the underlying mechanisms in uncomplicated DD (UDD). While an increase in the number of $CD68^+$ macrophages was observed in UDD, no change was observed in mast cells and $CD3^+$ T lymphocytes.[86,87] Although overexpression of TNF-α and IL-6 in UDD was observed, another study reported higher levels of IL-10 in the presence of UDD than in the healthy controls, but this finding was not of any statistical significance.[88-90] Mucosal TNF-α level were found to decrease in remission.[91] In the same study, levels of tissue inflammatory markers such as basic fibroblast growth factor (b-FGF) and syndecan-1 (SD1) were also high in UDD.

It can be inferred from the available information that role of inflammation in diverticulitis comes mainly from genetic studies. These studies cover SNP rs7848647, increased levels of TNF-like cytokine 1A (TL1A)/TNF superfamily member 15 (TNFSF15) genes.[92] Other SNPs also include SNP rs4662344 of the Rho-GTPase-activating protein 15 (ARHGAP15) gene, which plays an important role in selective neutrophil functions.[93] Compared to patients with recurrent diverticulitis, patients with complicated diverticulitis had higher levels of $CD68^+$ and $CD163^+$ macrophages in the biopsy testing of the samples taken from their colon.[86]

Studies have shown that microbiota may play a role in the transition from diverticulosis to UDD or diverticulitis but not in their pathogenesis.[83] *Pseudobutyrivibrio, Bifidobacterium*, and *Christensenellaceae* families were found

in high proportion in the feces of patients with recurrent acute diverticulitis.[94] The same study explored their relationship with the symptoms of patients with UDD and found that the excessive presence of *Ruminococcus* and the low presence of *Roseburia* were associated with the severity of bloating, and the excessive presence of *Cyanobacterium* was associated with the intensity of pain. The level of *Clostridium cluster IV, Fusobacterium*, and *Lactobacillaceae* were found to be lower in patients with UDD than in the control group of healthy individuals.[87] It has been shown that *Bifidobacteria*, especially *Bifidobacterium longum*, were found more in the colonic mucosal samples of patients with acute diverticulitis.[95]

IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is a functionally recognized disorder in which affected individuals complain of abdominal discomfort, bloating, pain, altered bowel movements (diarrhea, constipation, or both). The role of inflammation in IBS is questionable, but the information confirming its role is increasing. Two polymorphisms (SNPs rs4263839 and rs6478108) in the TNFSF15 gene, a proinflammatory cytokine gene, have been found to be associated with an increased risk of IBS. In the same meta-analysis and systemic review, SNP rs1800896 polymorphism for IL-10, an anti-inflammatory cytokine, was found to be associated with a decreased risk of IBS.[96] A low level of inflammation that stimulates the visceral nerves and causes dysmotility has also been reported.[97] Increased expression of the Prostaglandin E2 gene and increased protein expression were found in mucosal biopsies of patients with IBS with comorbid diarrhea. In addition, higher levels of IL-6, IL-8, and TNF-α were found in the serum of patients with IBS.[98,99] In a case control study, the use of antibiotics was found to enhance the risk of IBS.[100] There appears to be a link between the change in microbiota and the development of IBS. Compared to healthy individuals, patients with IBS were reported to have lower microbial diversity and higher Firmicutes/Bacteroidetes ratio.[101] It has been shown that microbial strains similar to *Methanobrevibacter smithii* can increase methane gas production, affect intestinal motor activity, and cause constipation. Additionally, prescribing patients antibiotic combinations to eradicate the breathe methane can improve constipation-dominant symptoms in IBS.[102] It has also been reported that colonic mast cells are increased in patients with IBS.[103] However, more data are needed to establish the relationship between IBS and inflammation.

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Inflammation in Rheumatoid Arthritis

Romatoid Artritte İnflamasyon

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ABSTRACT Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic and autoimmune disease. RA usually affects peripheral small joints symmetrically. It affects approximately 1% of population, and generally women are two to three times more likely to develop RA than men. Although its etiology is not clear, it results from interaction between genes and enviroment, which leads to breakdown of immune system, synovial inflammation, progressive cartilage and bone destruction. In preclinical RA period, RA associated antibodies such as rheumatoid factors (RFs), anti-citrullinated protein antibodies (ACPAs) and multiple inflammatory cytokines can be increased in the peripheral blood before the clinical arthritis. There are major genetic associations with the human leukocyte antigen (HLA) locus, for example several alleles of HLA-DRB1 are releated with high risk for antibody positive RA. In addition, epigenetic modification such as DNA methylation, histone modification affect the characteristic and function of the genes. On the other hand, application of cellular profiling techniques including single-cell transcriptomic and spatial transcriptomics has revealed new pathogenic cell types in RA synovial tissues. Finally, we discussed advances in genetics, epigenetics and novel biomarkers in the pathogenesis of RA.

Keywords: Rheumatoid arthritis; inflammation; epigenetic

ÖZET Romatoid artrit (RA) kronik, inflamatuar, sistemik ve otoimmün bir hastalıktır. Sıklıkla periferik küçük eklemleri tutar. Toplumda yaklaşık %1 oranında görülür ve genellikle kadınlarda erkeklerden 2-3 kat daha fazla görülür. Etiyolojisi bilinmemekle beraber, genlerin ve çevresel faktörlerin etkileşiminden kaynaklanır, bu da bağışıklık sisteminin bozulmasına, sinovyal inflamasyona, ilerleyici kıkırdak ve kemik hasarına yol açar. Preklinik RA döneminde, artrit gelişiminden önce periferik kanda romatoid faktörler (RFs), anti sitrüllenmiş protein antikorlar (ACPAs) gibi otoantikorlar ve birçok inflamatuar sitokin düzeyi artmış bulunabilir. İnsan lökosit antijen [human leukocyte antigen (HLA)] lokusları ile RA gelişimi açısından majör genetik ilişkiler mevcuttur. Örneğin HLA-DRB1 alelleri antikor pozitif RA gelişimi açısından yüksek riskli bulunmuştur. Ek olarak, DNA metilasyonu, histon modifikasyonu gibi epigenetik modifikasyonlar genlerin karakteristiğini ve fonksiyonunu etkilemektedir. Öte yandan, tek hücreli transkriptomik ve uzaysal transkriptomik gibi hücresel profilleme tekniklerinin uygulanması RA'lı sinovyal dokularda yeni patojenik hücre tiplerinin ortaya çıkmasını sağlamıştır. Sonuç olarak RA patogenezindeki genetik, epigenetik ve yeni biyobelirteçlerdeki ilerlemeleri ele aldık.

Anahtar Kelimeler: Romatoid artrit; inflamasyon; epigenetik

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory, autoimmune disease that mainly affects the synovial joints. The disease might cause joint damage, irreversible disabilty, loss of physical function, and inability to carry out activities of daily living if inadequately treated.[1] In addition uncontrolled inflammation may have other health risks and some accompanying chronic diseases such as osteoporosis, cardiovasculer diseases and even malignancies (eg, lymphoma and lung).[2-4] Typical presentation is usually insidious, with the predominant symptoms including pain and swelling of small joints of hands and feet and morning stiffnes.[5] Synovitis is generally symmetrical and affects small joints such as wrists, metacarpophalangeal

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(MCP), proximal interphalangeal (PIP). Other synovial joints like shoulders, elbows, knees and ankles are also commonly affected.[6,7] Prevalence of the disease in white individuals is in the range of 0,5-1% in the United States and Northern European countries and the annual incidence is estimated to be approximately 40 per 100.000 persons.[8-10] However, in some populations, such as Pima Native Americans, the incidence and prevalance of RA is much greater, which rates are up to 10 times higher than those of most population groups.[11] RA generally occurs in middle-aged and older people peaking between the ages of 65 and 80 years. RA is much more common in women and incidence rates are nearly twice as high when compared with males.[8,10]

Although the etiology of RA is still unknown, several risk factors including genetics, epigenetic modifications, environmental factors and female sex have been identified in epidemiologic and related studies in RA. However, many of them have not been externally validated and appear to have small effect size.[1]

RA is characterized by synovial inflammation and hypertrophy around synovial joints and can cause subsequent tissue damage. One of the hallmarks of RA pathogenesis is abnormal humoral and cellular immune responses. Cytokines and chemokines released by macrophages regulate osteoclast activity and cause cartilage and bone destruction.[12] Another important hallmark of RA is the presence of autoantibodies, particularly rheumatoid factors (RFs), and antibodies directed against collagen type 2.[13] Anti-citrullinated protein antibodies (ACPAs) is as sensitive and much more specific serological markers of RA. ACPAs can be superior alternative of the RF test for the diagnosis of RA. Frequently before the onset of RA symptoms and signs, ACPAs are present and they might predict the disease development. So that in the early course of the RA, ACPAs are valuable for the diagnosis.[14]

RA is complex genetic disease and the major histocompatibility (MHC) genes human encoding human leukocyte antigen (HLA) are the most important genes, besides many other genes also contribute to the disease severity.[15] Epigenetic modifications like DNA methylation and histone acetylation might also contribute to developing RA[16]

In this section we will review the issues that cause inflammation in rheumatoid arthritis.

PRECLINICAL RHEUMATOİD ARTHRITIS (RA)

In most patients immune abnormalities are detected prior to the development of clinical manifestations of RA, in other words the pathogenesis of RA begins years before clinical disease, that is called preclinical RA.[17] During this period, autoantibodies like ACPAs and RFs can appear more than 10 years prior to the onset of joint inflammation which pointing out to the immune activation.[17] Other antibodies against carbamylated and acetylated proteins might also occur.[18,19] In addition to autoantibodies, levels of multiple cytokines, chemokines and C-reactive protein, which emerges systemic inflammatory response, might increase in the preclinical phase.[20]

The development of RA occurs with the impact of genetic and environmental factors but the contribution of the environmental factors is not completely understood. Nevertheless, some stressors, for example smoking and microbiota can trigger citrullinization by inducing the peptidyl arginine deiminases (PAD).[21] After citrullination and other post-translational modifications, the altered peptides bind to MHC protein heterodimers, mainly those containing the shared epitope. This leads to antigen presentation to T cells and stimulate B cells to synthesize some antibodies that recognizing self proteins, such as RF and ACPAs.[22,23] However, the presence of ACPAs alone is not adequately to cause synovitis. An additional hit including complement activation, microvascular insult and immune complex formation is required to initiate clinical synovitis.[24]

GENETICS

It was shown that RA has a strong genetic component. In twin studies, the heritability of RA have been estimated approximately 60%, especially in patients who are positive for ACPAs. However, there is only 12-15% disease concordance between identical twins. This indicates that non-coding factors play a crucial role in the onset of RA.[1]

Recent genetic studies discovered more than 150 loci most of which associated with HLA class 2 show a very strong association with RA.[25] This class 2 HLA encode major histocompatibility complex (MHC) molecules that may contain the shared epitope (SE) which is a specific amino acid sequence at positions 69-74 of the beta chain commonly encoded by some alleles of the HLA-DR locus, especially HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*10 and HLA-DRB1*14:02 alleles. Those are significantly associated with the risk of developing RA. On the other hand, HLA-DRB1*13 alleles are thought to be protective against RA.[26] Therapeutic agents can target many of the proteins encoded by those genes. Outside of

the HLA locus some associations are detected at PTPN22, CTLA4 and PADI4 genes.[27,28] However, non-HLA associations is far lower in comparison to HLA-DRB1 associations.

Genetic differences between ACPAs (+) and ACPAs (-) RA patients have been showed, such as AFF3, CD28 and TNFAIP3 are found in only ACPAs (+) RA, PRL and NFAI are found only in ACPAs (-), whereas variants in HLA-DRB1, PTPN22, ANKRD55, IL6ST and BLK are associated with both ACPAs (+) and ACPAs (-) RA.[29] In addition variants in HLA-DRB1 differs between seropositive and seronegative RA. For example, HLA-DRB1*15, HLA-DRB1*04, HLA-DRB1*10 are associated with seropositivity (ACPAs) whereas HLA-DRB1*03 is associated with seronegativity.[26] Additionally, some RA susceptibility genes including HLA-DRB1, GRZB, MMP9, IL2RA and SPAG16 are found to be associated with severity of the disease.[30]

Interaction between cigarette smoking and SE alleles was detected previously.[31] Exposure to textile dust and alcohol consumption was also found to be interact with SE alleles.[32] In addition, GSTT1 and HMOX1 genes which are located at choromosome 22 were identified to increase risk of RA via the interaction with smoking.[33]

In a large-scale meta-analysis, including genome-wide associations studies (GWAS) was discovered 11 novel loci associated with RA.[34] The data were retrieved from previous GWASs in European, Japanese and Korean populations. In total 311.292 individual (22.628 RA, 288.664 control) included in the study.[35-37] The authors detected 11 novel loci including DGUOK-AS1, DAP, BAD, TPCN2, LOC107984408, LOC105369698, IQGAP1, PRKCB,ZNF689, C20orf181 and SMC1B as well as known RA associations such as PTPN22, CTLA4 and PADI4 in 71 non-MHC loci. The strongest genetic association were found HLA-DRB1 loci, as already known.[34] RA heritability were assessed on binding sites of 161 transcription factors (TFs) and it was significantly enriched in variants within binding sites of 29 TFs. Among those 12 showed especially large heritability enrichment. These 12 TFs have been significantly associated with T cell receptor (TCR) signaling mediated by mitogen-activated protein kinases, nuclear factor-kappa B and nuclear factor of activated T-cells, which reinforce the importance of CD4 (+) T-cell activation in RA pathogenesis. This suggest that variants associated with RA may play allele specific transcription regulatory role in CD4 (+) T cells.[38,39]

EPIGENETICS

Epigenetic changes in the expressions and functions of the genes may result in heritable phenotypes, although the DNA sequences remain unchanged. To date, three kinds of epigenetic modifications is known. First, elective transcription and expression regulation of some genes mediated by DNA methylation via chromatin remodeling, and genomic imprinting. Second one is post-translational modifications of proteins, such as histone methylation and acetylation. The last one is posttranscriptional regulation of genes, such as noncoding RNA, miRNAs, antisense RNAs, introns and riboswitches in the genome.[40]

The most commonly existing postreplication DNA modification in mammals, is DNA methylation. It is also one of the most broadly investigated epigenetic modifications.[41] Approximately 1046 DNA methylation sites are shown closely associated with the pathogenesis of RA.[42] DNA methylation modification in synovial fibroblasts and peripheral blood mononuclear cells (PBMCs) of RA patients is largely as hypomethylation of genomic DNA.[43] Hypomethylation in the promoter region is related to gene expression and activation of transcriptional activity, while hypermetylation promotes gene silencing or gene inactivation.[44] Demethylation of IL-6 and IL-10 genes contribute to the increase in cytokine levels and disease progression, and promotes the activation of fibroblast like synovial cells (FLSs).[43] Inhibitiors of DNA demethylation suppress the activation of FLS and the release of cytokines and chemokines. In general, methylation pattern may be a promising biomarker for prediction of outcomes and therapeutic monitoring of RA.[16]

The other epigenetic change is histone modification which is a post translational modification of a specific site on histones in chromatin including acetylation, methylation, phosphorylation and ubiquitination. Among these, acetylation is the most common histon modification. HDACs are thought to play an important role in silent regulation or the activation of proinflammatory genes. Inhibitors of HDACs are often used to examine the pathogenesis of RA.[16] In RA patients, the activity and expression of class 1 HDACs are found to decrease in PBMCs. This causes to the hyperacetylation and promotes proinflammatory process, finally leads to initiation of RA. Thus, activity of the HDACs and histone acetylation status in PBMCs can be potential biomarkers for the disease activity and promising target for the treatment of RA patients.[45,46]

Another posttranslational modification is ubiquitination. The main role of ubiquitination is protein degradation and its functional regulation. IL-1β increase the ubiquitin specific protease 5 (USP5) levels in FLS, and overexpression of UPS5 importantly stimulates activation of the related nuclear factor kappa B (NF-kB) signaling pathway and the production of proinflammatory cytokines.[47]

miRNAs which are about 22 nucleotides long and they are endogenous noncoding single stranded small RNAs. They act as a posttranslational suppressor of the gene leading to its translational suppression and degradation. miRNAs regulate about 30% of all gene expression and translation types essential for life process of cellular metabolism, differentiation, proliferation, inflammation and apoptosis. They participate in epigenetic regulation by changing histone modifications and regulating the levels of DNA methylation. This might have a role in the onset and progression of RA.[16]

BIOMARKERS

NON-ANTIBODIES BIOMARKERS IN RA

A biomarker is defined as a characteristic, which is objectively measured and evaluated as an indicator of normal biological as well as pathologic processes, or as a response to therapeutic intervention. However, most of biomarkers do not exactly fulfill the definition. They can be used for diagnosis, prediction of the prognosis of a particular disease, or response to treatment.[48,49] While transcriptomic and cellular technologies formerly have focused on preselected cell types, examined whole tissues rather than single cells on RA joint tissue, nowadays, due to recent advances in cytometry, transcriptomic profiling and spatial transcriptomics, researchers might have a chance in studying cellular and molecular heterogeneity in blood and RA synovium. Combining highly purified proteolytic enzyme with mass cytometry, 18 unique cell states was identified in RA synovia.[50]

The efficacy of B cell depletion treatments supports a role for B cells in pathogenesis of RA.[51] Activated memory B cells population exhibit in the peripheral blood of RA patients and four distinct B cell states including plasmablasts, naive B cells, memory B cells and autoimmune associated B cells (ABCs) was identified. ABCs are highly expanded with active disease in RA patients.[50] T cells are also important mediators of chronic inflammation in RA synovial tissue. However, until recently which T cell populations drive B cell aggregation and proliferation was not known in RA synovium. CD4 (+) PD-1 CXCR5 (-) T cells, that is called peripheral helper T (Tph), induce memory B cell differentiation into plasma cells have been described. These cells are located adjacent to B cells within lymphoid aggregates in RA synovial tissue.[52] In addition, CD8 (+) T cell subsets characterized by differential expression of GZMK and GZMB also revealed.[50]

There are two main cell types including fibroblasts and macrophages in the synovium. Two important inflammatory monocyte population were identified among the myeloid compartment in inflamed synovium.[50] While one of them is characterized by high expression of IL-1β and HBEGF, the other is characterized by an interferon activation signature. IL-1β, HBEGF and inflammatory macrophages promoted fibroblast invasiveness *in vitro*. This suggests a potential role for IL-1β, HBEGF and inflammatory macrophages in driving fibroblast mediated joint destruction.[50,53] On the other hand, some macrophages express MERTK and TREM2 marker during disease remission. MERTK and TREM2 might suppress inflammation and induce a reparative response in synovial fibroblasts and a reduced frequency of these macrophages was found with an increased risk of flare in disease remission, which means they may play a role in sustaining remission.[54]

Synovial fibroblasts are mesenchymal cells and secrete proteoglycans to promote normal joint functions in the joint. However, synovial fibroblasts, especially CD90 (+) ones, take on pathological roles by recruiting leukocytes, promoting neoangiogenesis and degrading cartilages, which contribute to joint damage in RA synovia. Recently, vascular endothelium derived Notch signaling that is a key driver of CD90 (+) fibroblast differentiation was identified and inhbition of this Notch receptor signaling suggests a significant role for driving CD90 (+) fibroblast differentiaton.[55]

Although previously, endothelial cells that line blood vessels have been perceived as passive, structural units, recent studies have showed that specialized endothelial cells was found in niche tissue microenvironments and actively orchestrate tissue remodeling via paracrine secretion of morphogenic signals also termed angiocrine factors.[56] Angiogenesis, the formation of new blood vessels, is crucial for supplying oxygen and nutrients to immune and stromal cells in inflamed tissue of RA synovium. Hypoxia induces the production of proangiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and placenta growth factor (PGF). This induce angiogenesis and remodeling of the surronding tissue in inflamed tissue.[57] Analysis of synovial endothelial cells from RA synovium by unbiased scRNAseq showed that arterial endothelial cells can be identified by the expression of PODXL and NOTCH4,

while venous endothelial cells are characterized by the expression of CLU and ACKR1. Addionally, beyond the angiocrine factors, synovial endhotelial cells induce many cytokines, chemokines and growth factors.[58]

ANTIBODY-DERIVED RA BIOMARKERS

Rheumatoid Factor (RF) is the first biomarker of RA. The sensitivity of RF IgM is between 70-75%, specifity is between 80-85%.[59] RF is an autoantibody targeting the Cγ2-Cγ3 domain in the Fc region of IgG and is one of the commonly used biomarker for RA diagnosis.[60] However, as negative results of RF test do not rule out RA, as positive results can occur in other disease. RF is normally used for diagnosis of RA, not monitoring of RA. RF is more strongly related with joint structural damage, activity and progression of the disease than ACPA.[61] The major processes that cause to formation of autoantibodies are posttranslational modification of proteins such as citrullination, carbamylation, glycation.[48] These posttranslationally modified proteins are produced via enzymatic or chemical process and their main source might originate from neutrophils undergoing NETosis process during viral or bacterial infection. Before the NETosis, chromatin is decondensed with the help of demethylation and PAD-4 mediated deamination.[62] The primer area of NETosis is periodontal dysbiosis and mucosal surface which might cause to citrullination of some proteins. Porphyromonas gingivalis has its own PAD enzyme which may lead to citrullination.[63] On the other hand, Aggregatibacter actinomycetemcomitans produces leukotoxin causing to hyperactivation of citrullination enzymes in neutrophils.[64]

ACPAs are the second important diagnostic and the most specific biomarkers for the diagnosis of RA. The main posttranslational modification of ACPAs is citrullination. Smoking and periodontitis caused by Porphyromonas gingivalis can initiate citrullination.[65] Citrullinated peptides were recognised since 1960s and the first one was antiperinuclear factor (APF). After that, anti-keratin antibodies (AKA), anti-filaggrin antibodies (AFA), anti-mutated citrullinated vimentin (anti-MCV), antibodies against citrullinated alfa enolase peptide 1 (anti-CEP-1) and antibodies against citrullinated vimentin (anti-Sa) were identified. Today, ACPAs are used to describe the antibodies against various citrullinated proteins. Synthetic cyclic citrullinated peptides are used to recognize for ACPAs, in recent. They are called anti-cyclic citrullinated peptide antibodies and have 88-99% specificity.[66] ACPAs are identified in patients with cardiovascular disease (CVD) and interstitial lung disease (ILD), which are correlated with high mortality in RA patients.[67]

More than 90% of ACPA-IgGs harbor N-linked glycans in the antibody variable (V) domain, which are completely galactosylated and sialylated. In ACPA IgG (+) first degree relatives of RA patients who later developed RA, broad V domain glycosylation was shown before the onset of inflammatory arthritis. ACPA IgG V domain glycosylation had a positive predictive value of 76.% and had a negative predictive value of 78.6%, for predicting RA development. Thus, it might be a predictive marker in ACPA (+) people for RA development.[68]

Carbamylation is a chemical process, which affects lysine and causes to nonenzymatic posttranslational modifications of proteins. Antibodies against carbamylated proteins (anti-CarP) are seen approximately 43-45% in the serum of RA patients and approximately 16% of ACPA (-) RA patients have anti-CarP antibodies.[69] The sensitivity of anti-CarP is between 27-46%, the specifity is in the range of 89-97%.[65] The presence of anti-CarP is a strong predictor for the development of RA in patients who have pain or arthritis, even though they do not have RFs or ACPAs. Furthermore, these antibodies predict severe disease course and might be a prognostic biomarker, especially in ACPA (-) RA patients.[70]

Except these antibodies, the presence of other antibodies including antibodies against oxidized proteins, malondialdehyde-acetaldehyde (MAA) adducts, antibodies against peptidyl arginine deiminase, antiRA33 antibodies, anti G6PI antibodies, anti-BiP antibodies, anti-type 2 collagen antibodies and anti-gp39 antibodies are detected in RA patients.[48]

In conclusion, current application of cellular profiling technologies has given us an insight into the pathogenesis of RA, which contribute to therapeutic approaches. The autoantibodies are still the most frequently used biomarkers for diagnosis, as well as for prognosis. However, genetic and epigenetic advances are essential in understanding the pathogenesis of RA.

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Aging and Inflammation

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ABSTRACT Age-related immune system changes are called immunosenescence. Immunosenescence is characterized by an increased risk of inflammation, infection, malignancy, and autoimmune disorders. Older adults exhibit a chronic, sterile, low-level inflammatory process called "inflammaging". With aging, stem cells in the bone marrow decrease and their telomeres shorten. Moreover, acquired genomic or mitochondrial DNA defects accumulate and result in decreased regeneration capacity of hematopoietic stem cells. In addition, pro-inflammatory markers such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and reactive oxygen species (ROS) levels increase with aging. Accumulation of senescent cells, production of leptin from adipose tissue, altered gastrointestinal flora, and underlying diseases may cause immune deficiency and susceptibility to infection. Nutrition, exercise, and vaccination come into prominence to slow the effect of the aging immune system.

Keywords: Aging; immune system; stem cell; telomere; DNA damage

ÖZET Yaşa bağlı bağışıklık sistemi değişikliklerine bağışıklık yaşlanması denir. İmmünosenesens, artmış inflamasyon, enfeksiyon, malignite ve otoimmün bozukluklar riski ile karakterizedir. Yaşlı yetişkinler, "inflammaging" adı verilen kronik, steril, düşük düzeyde bir iltihaplanma süreci sergilerler. Yaşlanmayla birlikte kemik iliğindeki kök hücre rezervi azalır ve bu hücrelerin telomerleri kısalır. Edinilmiş genomik veya mitokondriyal DNA kusurları birikir ve hematopoietik kök hücrelerin rejenerasyon kapasitesinin azalmasına neden olur. Ayrıca interlökin 6 (IL-6), tümör nekroz faktörü-α (TNF-α), C-reaktif protein (CRP) ve reaktif oksijen türleri (ROS) seviyeleri gibi proinflamatuar belirteçler yaşlanmayla birlikte artar. Yaşlanmış hücrelerin birikmesi, yağ dokusundan leptin üretimi, değişen gastrointestinal flora ve altta yatan hastalıklar, immün yetmezliğe ve enfeksiyona yatkınlığa neden olabilir. Yaşlanan bağışıklık sisteminin etkisini yavaşlatmak için beslenme, egzersiz ve aşılama ön plana çıkmaktadır.

Anahtar Kelimeler: Yaşlanma; immün sistem; kök hücreler; telomer; DNA hasarı

Aging is a complex process arising from a combination of environmental, genetic, and epigenetic factors. It develops under the influence of extrinsic and intrinsic factors and is progressive. Although there are more than 200 theories about aging, there is no definitively accepted theory. Two of the most accepted of these theories are the cellular theory of aging and inflammation. With advancing age, changes occur not only in the immune system, but in all systems These changes are called aging of the immune system (immunosenescence). Immune aging does not affect all components of the immune system equally. An important concept in immune aging is the loss of regulation of inflammatory processes. Immunosenescence is characterized by an increased risk of inflammation, infection, malignancy, and autoimmune disorders. Older adults exhibit a chronic, sterile, low-level inflammatory process called "inflammaging".[1] The dysregulation of the inflammatory process, which develops with aging, is found in almost every living organism. Inflammation plays a role against pathogens and helps in

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the maintenance of homeostasis. This temporary inflammatory state is essential for wellness. Since the cause (aging) that triggers the inflammatory reaction is not eliminated, the inflammation becomes chronic and begins to damage the organism. This inflammation may cause many geriatric syndromes or contributes to the process, causing morbidity and mortality.

Inflammaging is the chronic activation of the innate immune system in which the macrophage is at the center.[2] In addition, inflammation is involved in various cellular and molecular mechanisms such as cellular aging, mitochondrial dysfunction, autophagy and mitophagy, inflammasome activation, dysregulation of the ubiquitin-proteosome system, activation of DNA damage response, and dysbiosis (Figure 1).[2-4]

All immune cells originate from hematopoietic stem cells in the bone marrow. With aging, stem cells in the bone marrow decrease and their telomeres shorten. [4] In addition, advancing age brings with it acquired genomic or mitochondrial DNA defects accumulate. As a result, the regeneration capacity of hematopoietic stem cells decreases. Decreased regenerative capacity in stem cells becomes more important in cases of disease and physiological stress. Hormones, inflammatory cytokines, and changes in antioxidant levels with aging make it difficult to maintain homeostatic balance. It is well known that pro-inflammatory markers such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and reactive oxygen species (ROS) levels increase with age.[5]

IL-6 is the cytokine most associated with aging.[6] Based on data from approximately 4,000 participants in the PolSenior and Framingham Heart studies, serum level of IL-6 is a valuable predictor of mortality in older adults.[7,8] IL-6 is the most important inducer of CRP, which has been identified in the serum of patients with *Streptococcus pneumoniae* infection and is currently used as a nonspecific inflammation biomarker.[9] In the PolSenior study, the increase in CRP level was related to increased mortality risk similar to IL-6. Pentraxin-3 (PTX3) is synthesized locally from the liver in response to IL-6 and locally from myeloid and stromal cells in response to primary proinflammatory cytokines such as IL-1β and TNF-α.[10] In cohort studies, circulating PTX3 and CRP levels might be correlated with age and were likely indicators of chronic low-grade inflammation.[11]

Despite all this knowledge, the origin of cytokines and chemokines in this chronic inflammatory process is not entirely clear. It has been shown that in diseases where the

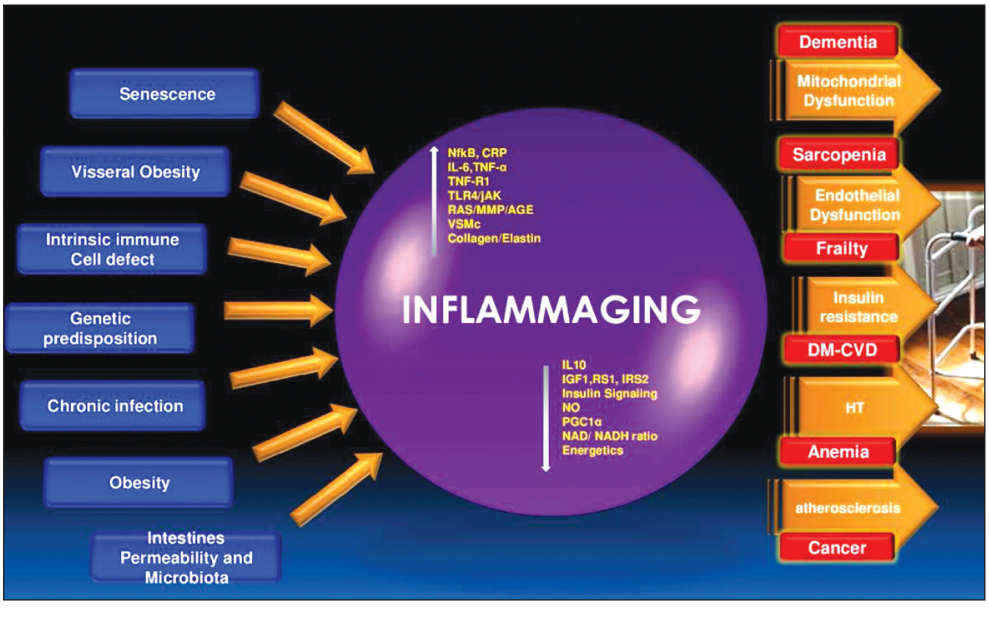


FIGURE 1: Inflammaging: potential causes.

CRP: C-reactive Protein, CVD: Cardiovascular Disease, DM: Diabetes Mellitus, HT: Hypertension, IGF-1: Insuline like growth factor-1, IL-6: Interleukin 6, IL-10: Interleukin 10, IRS1:Insulin receptor substrates-1 IRS2: Insulin receptor substrates-2, NAD/ NADH: Nicotinamide adenine dinucleotide/NAD/NADH: Nicotinamide adenine dinucleotide/Nicotinamide adenine dinucleotide + hydrogen, NfkB: Nuclear factor kappa-light-chain-enhancer of activated B cells, NO: nitrous oxide, PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, RAS/MMP/AGE: renin-angiotensin system/ Matrix metalloproteinase/Advanced glycation end products, TLR4/jAK: Toll Like reseptör/janus kinase, TNF-α: Tumor necrosis factor -α, TNF-R1: Tumor necrosis factor receptor 1,VSMc: Vascular smooth muscle cells.

immune system is rapidly aging, as in HIV infection, microbial products in the intestine may cross into the circulation and tissues more easily.[12] With aging, the gastrointestinal tract flora changes, intestinal and vascular permeability increases. These changes are associated with increased IL-6 and IL-8 serum levels.[13] Another reason may be senescent cells whose proliferation has stopped and their functionality has changed.[14] Debris and immunoglobulin accumulation due to age-related deterioration in elimination systems trigger the activation of the immune system, leading to permanent inflammation. Glycosylation disorder (a galactosylated protein structure), which is seen in many autoimmune/inflammatory diseases such as progeria disease, disrupts the Fc structure of immunoglobulin G (IgG) and causes a proinflammatory state, including the lectin pathway of complement. N-glycans devoid of galactose residues detected in human studies comparing healthy older individuals and their children and in calorie-restricted mouse models were shown as new biomarkers of aging.[15-19] However, telomerase shortness, genotoxic stress, and increased proinflammatory cytokines in senescent cells result in the activation of p53 tumor suppressor and p16 cyclin-dependent kinase inhibitor genes.[20] As a result of the irreversible arrest of the cell cycle, a cell with a senescence-associated secretory phenotype (SASP) is formed. SASP causes the release of inflammatory cytokines and low-grade inflammation, and the microenvironment of this cell is also disrupted.[21,22] Although SASP may promote tissue repair and remodeling, which requires a controlled inflammatory response, it may cause pathological consequences when present chronically.

This phenotype has been shown as the main source of inflammation in diseases that increase with age, such as atherosclerosis, malignancy, and type 2 Diabetes Mellitus. Also, in animal studies, clearance of senescent cells has shown a reduction in age-related changes.[23,24]

Increased procoagulant state and fibrinolysis activity in older adults have been associated with increased inflammation via the protease-activating receptor (PAR).[25] It is known in studies that the plasma concentrations of coagulation factors I, V, VII, VIII, and IX increase in healthy people with aging.[26] However, factor X is locally synthesized at high levels in atherosclerotic plaques, smooth muscle cells, and inflammatory cells.[27,28] Although the mechanism is not clear, activation of the coagulation cascade following fibrinogen activation may increase thrombosis, and increased levels of coagulation factor Xa and thrombin may increase inflammation via PAR½.[29] In addition, it has been shown that coagulation factors Xa and PAR ½ increase the expression of insulin-like growth factor binding protein-5 (IGFBP-5).[29] There is growing evidence that IGFBP-5 increases the production of reactive oxygen radicals by various mechanisms, causes DNA damage, and regulates aging, especially in fibroblasts. In line with these data, this mechanism may be a reason for the increase in inflammation caused by coagulation factors that change with age.

Adipose tissue has also been shown to play an important role in inflammation. It has been shown that adiponectin regulates the production of anti-inflammatory cytokines in leaner individuals, and induces the production of leptin, TNF-α, IL-6, and IL-12 secreted from systemic inflammatory conditions and excess adipose tissue.[30-32] The normal aging process results in an increase in adipose tissue. Excess adipose tissue is associated with a proinflammatory state in the mouse.[33] It has been suggested that increased adipose tissue is the cause of high inflammatory cytokines observed in obese individuals.[34] The fact that older individuals who exercise regularly and are thinner have fewer aging T cells and lower pro-inflammatory cytokine levels support this suggestion.[35] In addition to obesity, cardiovascular and neurodegenerative diseases are examples of inflammation-related diseases.[5,36]

Besides these immune changes, which are the "normal" process of aging, secondary causes are effective on the immune system in older adults. Underlying diseases such as malnutrition, malignancies and malignancy treatments, immunosuppressive agents, Diabetes Mellitus, and uremia may cause immune deficiency and susceptibility to infection in the elderly with immunosenescence. A low-grade increase in circulating TNF-α, IL-6, soluble IL-2R receptor (sIL-2R), and CRP levels is observed in age-related diseases, involving both the innate and adaptive immune systems. The effects of inflammatory mediators on survival are independent of existing morbidity and other mortality risk factors (smoking, blood pressure, physical exercise, total cholesterol, comorbidity, body mass index, and anti-inflammatory drug intake). In addition, these molecules may serve as sensitive markers for preclinical conditions in elderly populations.[37,38]

Studies are showing that adequate nutrition and regular, moderate exercise are important for immunity; however, there is no approved pharmacological treatment against immune aging.

Nutrition: Available data indicate that vitamins (A, D, E, B6, B12, folate, and C) and elements (selenium, zinc, copper, and iron) are essential for normal immune function.[39,40] However, there is no data to suggests that supplementation could improve immune function beyond reaching recommended daily nutrient amounts. It would be

appropriate to replace these nutrients in clinical and laboratory terms.

Smoking: Of the 1017 oxidant molecules in smoke, 1014 are reactive oxygen species.[41] Nitric oxide and other free radicals in smoke induce inflammation by increasing the production of several proinflammatory cytokines such as IL-6, TNF-α, and interleukin-1β (IL-1β).[42] Consequently, cessation of smoking is one of the most important steps to be taken against aging.

Exercise: While there is some evidence that prolonged, moderate exercise may improve immune function in older adults vigorous exercise may also be immunosuppressive.[43,44]

Vaccination: Impaired vaccine response in elderly individuals may be due to decreased quantitative and functional memory T cells and decreased functional vaccine-specific antibodies.[45] Vaccines to protect against tetanus, diphtheria, pertussis, influenza, pneumococcal infections, and zoster are especially important for individuals older than 65 years.

While the impact of the SARS-CoV-2 pandemic on the whole world, we have known that the infected older adults have more severe diseases and progress with higher mortality and morbidity. Age-related respiratory system changes, such as a decrease in mucociliary activity, decrease in surfactant production, increase in the level of ACE 2 (the receptor of SARS-CoV-2) and the age alone is an important risk factor for both severe disease and death.[46-48] The importance of SARS-CoV 2 vaccination in elderly individuals should not be underestimated.

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Movement and Inflammation

Hareket ve İnflamasyon

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ABSTRACT The concept of movement includes physical activity and exercise. Physical activity is any bodily movement that increases energy expenditure above resting levels. Exercise is part of physical activity and is a planned and structured behaviour. Anti-inflammatory effects of exercise have focused on three possible mechanisms: the reduction in visceral fat mass; increased production and release of anti-inflammatory cytokines from contracting skeletal muscle and reduced expression of Toll like receptors (TLRs) on monocytes and macrophages. Studies in the literature have demonstrated that chronic inflammation may increase the risk of disability and mortality even in people who do not have clinical disease. Regular exercise protects against diseases associated with chronic low-grade systemic inflammation. Exercise induced changes in inflammation can be categorized as acute effects and long-term effects of exercise. The pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) are released after physical activity of sufficient intensity, followed by the release of anti-inflammatory or regulatory cytokines. During exercise, IL-6 is the first detectable cytokine released from the contracting skeletal muscle cells into the blood. Muscle-derived IL-6 increases with exercise. The duration of exercise is the single most important factor that determines the magnitude of the systemic IL-6 response. Acute exercise bout initiates a complex, time dependent cascade of inflammatory events, which depends largely on the mode, intensity, duration of the exercise bout. Regular physical exercise training may be considered a long lasting anti-inflammatory therapy, after the acute inflammatory actions are resolved. The long term effects of exercise on reducing inflammation are thought to be mainly mediated by reductions in the size of adipose tissue.

Keywords: Exercises; inflammation

ÖZET Hareket kavramı içinde fiziksel aktivite ve egzersiz vardır. Fiziksel aktivite istirahat düzeyinin üzerinde enerji harcamasını artıran tüm vücut hareketleridir. Egzersiz fiziksel aktivitenin bir kısmıdır, planlı ve yapılandırılmış davranıştır. Egzersizin anti-inflamatuar etkileri üç olası mekanizmaya odaklanmıştır: viseral yağ kütlesindeki azalma; kasılan iskelet kasından anti-inflamatuar sitokinlerin salınımı; monositler ve makrofajlar üzerindeki Toll like reseptörlerin (TLR'ler) azalmasıdır. Çalışmalar, kronik inflamasyonun klinik hastalığı olmayan kişilerde bile özürlülük ve ölüm riskini artırabileceğini göstermiştir. Düzenli egzersiz, kronik düşük dereceli sistemik inflamasyon ile ilişkili hastalıklara karşı koruma sağlar. İnflamasyonda egzersizle oluşan değişiklikler akut ve uzun süreli etkiler olarak ayrılabilir. Yeterli yoğunluktaki fiziksel aktiviteden sonra proinflamatuar sitokinler (TNF-α, IL-1β ve IL-6), ardından anti-inflamatuar veya düzenleyici sitokinler salınır. Egzersiz sırasında IL-6, kasılan iskelet kası hücrelerinden kana salınan ilk saptanabilir sitokindir. Kaslardan salınan IL-6 egzersizle artar. Egzersizin süresi sistemik IL-6 yanıtının büyüklüğünü belirleyen en önemli faktördür. Akut bir egzersiz, büyük ölçüde egzersizin moduna, yoğunluğuna, süresine bağlı olan karmaşık, zamana bağlı bir inflamatuar olaylar zincirini başlatır. Düzenli egzersiz eğitimi, akut inflamatuar eylemler çözüldükten sonra uzun süreli bir anti-inflamatuar tedavi olarak kabul edilebilir. Egzersizin inflamasyonu azaltma üzerindeki uzun vadeli etkilerine, esas olarak yağ dokusunun boyutundaki azalmaların aracılık ettiği düşünülmektedir.

Anahtar Kelimeler: Egzersiz; inflamasyon

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The term movement includes the concepts of physical activity and exercise. Physical activity is defined as any bodily movement produced by skeletal muscles, which results in energy expenditure above resting levels.[1] Physical activity includes occupational activities (e.g., work, domestic cleaning), transport (e.g.,walking or cycling to work) and leisure-time activities (e.g.,playing, dancing).[2,3]

Exercise is a subset of physical activity that is planned, structured, and repetitive activity and has as a final or an intermediate objective the improvement or maintenance of physical fitness.[4-6]

Exercise has anti-inflammatory effects, and therefore, in the long term, regular physical activity can protect against chronic non-communicable diseases, dementia and cognitive decline, among other diseases.[7-11]

Exercise and physical activity can also invoke anti-inflammatory actions independent of changes in fat mass inflammatory stimuli and contributes to regulation of inflammatory reactions, but its measurement is frequently used as a biomarker for inflammation.[12,13]

Due to enhanced musculoskeletal function, cardiorespiratory and metabolic health, sleep, pain management, cognition, learning, memory and more, the practice of physical exercise represents a nonpharmacological primary clinical tool for the prevention of many diseases whose physiopathology is linked to acute or sustained immune system activation.[14] In general repeated moderate intensity exercise enhances the immune function response, reinforces the antioxidative capacity, reduces oxidative stress, and increases the efficiency of energy generation, therefore reducing the incidence of inflammatory diseases.[15,16] Acute bouts of physical exercise also regulate the immune response, i.e. by transiently redistributing immune cells to peripheral tissues, resulting in a heightened state of immunocompetence. The acute phase response is regulated by specific pro-inflammatory cytokines, predominantly IL-6. The effects of acute exercise on inflammation have been mainly investigated in the muscle by assessing muscle-derived cytokines termed “mykines”, while the long term effects of exercise on inflammation are mainly observed in adipose tissue with adipose tissue-derived cytokines termed ‘adipokines’.[17-21]

THE EFFECTS OF ACUTE EXERCISE ON INFLAMMATION

Physical exercise enhances energy expenditure through muscle contraction, in which energy (glucose and fatty acids) is used to generate mechanical forces and heat in muscle.

The muscles secret myokines, which are cytokines or peptides released by muscle fibers exert autocrine, paracrine or endocrine effects. Identified myokines include IL 6, IL 7, myostatin, LIF, BDNF, IGF 1, FGF 2, FSTL 1 and irisin.[22] Muscle contraction during exercise is now thought to be the main trigger for overexpression of IL-6. Exercise-induced IL-6 within the muscle, increases IL-6 in the circulation which then acts as a trigger to activate hepatic glycogenolysis and lipolysis; this occurs because energy within the muscle is quickly depleted with exercise, so this mechanism triggers pathways to provide additional energy to the exercising muscles.[9,15,16] However, this overproduction of IL-6 is not thought to induce inflammation. These observations suggest that exercise may acutely promote an anti-inflammatory environment within the human body.

THE EFFECTS OF LONG TERM EXERCISE ON INFLAMMATION

It is well-established that obesity is a low-grade state with adipose tissue acting as an endocrine and paracrine organ responsible for stimulating the increase of inflammatory mediators. In support of this, the expression of inflammatory mediators, such IL-6 and TNF-α deriving from macrophages residing in adipose tissue cells, are overexpressed in overweight and obese compared with normal weight individuals, while inflammatory processes are activated early in the expansion and progression of adipose tissue. On the other hand, evidence suggests that exercise can have a significant effect in reducing the size of adipose tissue cells. On the other hand, exercises may promote an anti-inflamatory effects in different tissues.[6,22-24]

In general repeated moderate intensity exercise enhances the immune function response, reinforces the antioxidative capacity, reduces oxidative stress, and increases the efficiency of energy generation, therefore reducing the incidence of inflammatory diseases.[25,26]

The activation of the immune system results in the release of cytokines that can be classified into pro- (including IL-1, IL-8, TNF-α, IFNγ, VEGF, among others) or anti-inflammatory (i.e. IL-2, IL-4, IL-10, IL13) factors. Chronic physical exercise can guide the immune system response by favoring an anti-inflammatory status, which appears to be the key factor in improving health, mainly in chronic diseases.[27]

Anti-inflammatory effects of physical exercise;

The anti-inflammatory cytokines, including IL-10 and IL-6, belong to a group of regulatory mediators that govern the pro-inflammatory cytokine response mediated by IL-1β, IFN-γ, TNF-α, and others.

The anti-inflammatory effects of regular exercise may be mediated via both a reduction in visceral fat mass (with a subsequent decreased release of adipokines) and the induction of an anti-inflammatory environment with each bout of exercise.

The anti-inflammatory effects of physical exercise appear to be controlled by multiple mechanisms. Some of them are represented by (i) increased production of adrenaline, cortisol, growth hormone, prolactin, among others that have immunomodulatory effects by influencing leukocyte trafficking and functions ; (ii) visceral fat loss; (iii) improved levels of anti-inflammatory myokines by the working skeletal muscle, and (iv) diminishing the expression of TLRs in immune cells.[28]

1) Physical exercise-induced release of stress hormones: Adrenaline and noradrenaline are the fast-acting fight or flight hormones produced by the adrenal glands on direct signaling from the brain under a stressful stimulus. Cortisol is a steroid hormone also produced by the adrenal glands, not released instantly as adrenalin, but rather taking minutes since it involves a stepwise activation of the amygdala. Both, adrenaline and cortisol, rapidly increase during exercise and once released, adrenaline activates the sequential releases of corticotropin-releasing hormone by the hypothalamus, adrenocorticotropic hormone by the pituitary gland, and finally of cortisol by the adrenal glands. The responses induced by the release of adrenaline and cortisol typically are upstream of the immune system response.[25,26]

2) Physical exercise-induced anti-inflammatory effects on adiposity: Increased visceral adipose tissue (white adipose tissue; WAT) deposition is a risk factor for the developing of many chronic inflammatory conditions, including the pandemics of obesity, and T2DM, and the public health problems, chronic pain and cardiovascular diseases. WAT is known to produce many adipokines, including TNF-α, IL-6, IL-18, leptin and retinol-binding protein 4. Excessive WAT provokes the reduction of plasma anti-inflammatory mediators, leading to the development of a chronic low-grade systemic inflammatory state.[27,28]

3) Increased production and release of anti-inflammatory myokines from contracting skeletal muscle: Contracting skeletal muscle produces myokines, such as IL-6, IL-15 and irisin, which exert beneficial effects on the adipocyte metabolism.[22,29] It has been demonstrated that infusion of IL-6 in healthy individuals delays gastric emptying with positive effects on postprandial glycemia, increases glucose uptake and lipid mobilization and oxidation, reducing therefore the risk for metabolic disorders. IL-15 is also an anabolic factor present in the muscle and related with reduced WAT mass. The high expression of IL-15 prevents rodents from the visceral fat deposit by increasing lean body mass, and it has been shown to be increased in trained human muscle.[30,31] Therefore, either IL-6 and IL-15 have a significant role in lipid metabolism by preventing obesity development. Irisin, another myokine released into the circulation by exercise training, has demonstrated to induce thermogenic actions in adipose tissue in rodents and humans. Irisin has been recently shown to be linked to the exercise induced beneficial effects in the cardiovascular, digestive, immune and adipose systems.[32,33]

4) Reduced expression of TLRs in immune cells: Systemic inflammation is controlled by the activation of TLRs signaling. TLRs are highly conserved transmembrane proteins that play an important role in the detection and recognition of microbial pathogens, and they can also be activated by endogenous danger signals of tissue damage, such as heat shock proteins.[34,35] It has been demonstrated that physical inactivity correlates with augmented TLRs activation, and consequently, in the development of chronic non-communicable diseases. On the other hand, chronic exercise has been demonstrated to decrease cell-surface expression of TLRs on immune cells.[35,36]

ACUTE EXERCISE

The acute exercise bout initiates a complex, time dependent cascade of inflammatory events, which depends largely on the mode, intensity, duration, and familiarity of the exercise bout. The inflammatory mediators which regulate this response act upon various tissues, most notably the skeletal muscle. Here, the inflammatory cascade is characterized by an initial pro-inflammatory response (1.5-24 h after exercise) which is followed by an anti-inflammatory muscle regenerative response (24-72 h after exercise). Acute exercise also initiates “inflammatory” processes in the circulation, evident by significant rises in circulating myokines (i.e., IL-6) followed by a subsequent increase in circulating anti-inflammatory cytokines (IL-10 and IL-1RA). Acute exercise also acts on immune cell receptor presentation, evidenced by a significant reduction of TLRs on circulating monocytes 1.5 h after strenu-

ous exercise. It is currently unclear how an acute inflammatory response to exercise initiates long-term adaptive responses.[37-39]

EXERCISE AND CHRONIC INFLAMMATION

Studies have demonstrated that chronic inflammation may increase the risk of disability and mortality even in people who do not have clinical disease. For instance, for individuals with infection, C-reactive protein (CRP) levels may be 1000 fold higher than the standard values. CRP is a hepatic acute phase protein that is commonly used as a biomarker of systemic inflammation and risk factor for cardiovascular disease.[35,36,40,41] In addition, increased CRP is related to diabetes, heart failure, physical disability, and other diseases. Therefore, the action ways of diminishing chronic inflammation is critical for reducing the risk of inflammation associated diseases. Regular physical activity has the potential to improve chronic inflammation. Regular moderate exercise training has been shown to act in an anti-inflammatory fashion in a number of states where inflammation is chronic in nature including aging, obesity, metabolic disease, spinal cord injury, and stroke among others.[42-44]

DOSAGE OF EXERCISE AND INTRODUCTION OF ANTI-INFLAMMATORY RESPONSES

The exact dosage of exercise that can improve health outcomes, including reducing inflammation, is an ongoing area of investigation. It is exercise rather than habitual physical activity that has been the mainly focus with regards to inflammatory responses, since exercise seems to have a dose-dependent relationship with health outcomes and inflammatory mediators. For exercise, on the other hand, the American College of Sports Medicine (ACSM) has conducted substantial work in defining the beneficial exercise dosage, using the frequency, intensity, time and type (FITT) of exercise as determinants of dosage.[40,41]

It is agreed currently that, although a minimum dosage is required to increase cardiorespiratory fitness, the lack of consistent findings in this area is an issue of concern, and predominantly influenced by the initial level of fitness/conditioning of the exercising participants.[45,46]

Physical exercise as a non-pharmacological intervention for neuroprotection, the regular practice of physical exercise is known to be beneficial for the CNS health and function, counteracting age related cognitive decline and dementia risk in humans and rodents.[47] A great body of evidence has shown that exercise improves neurological impairments induced by brain injury and promotes functional recovery by preventing neuronal loss. Moderate physical exercise training has been shown to promote neuroplasticity, neurogenesis and neuroprotection, in addition to hippocampus dependent learning and memory. Neuroinflammation is a common hallmark in many disorders of the CNS, e.g., depression, Alzheimer's disease and Parkinson's disease, and it is responsible for the pathological reactive activation of glial cells (microglia and astrocytes).[30]

In animal studies, exercise training lowered adipose tissue inflammation, possibly due to an improvement in adipose tissue hypoxia, which is independent of its effect on weight loss. In addition, exercise training may reduce chronic inflammation by affecting muscle cytokine release, and reducing inflammatory cytokine production by endothelial cells and innate immune cells. Future studies are needed to better define the dose-response effects of exercise training on specific aspects of the inflammatory cascades (e.g.initiation, promotion, amplification and signalling), and to further investigate the tissue and cellular mechanisms by which exercise training reduces chronic, systemic inflammation in obese individuals.[48-50]

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Surgical Inflammation

Cerrahi İnflamasyon

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ABSTRACT Inflammation is the tisuue response to various dangerous and harmful internal or external stimuli. The effect of surgery could either be local at the site of surgical procedure or at a remote area in the body. Surgical interventions may lead to a systemic inflammation with different effects: secretion of proinflammatory cytokines, endothelial dysfunction, glycocalyx damage, activation of neutrophils, and ultimately tissue and multiple organ dysfunction.The factors determining the effects of inflammation after surgery depends on pro- and anti- inflammatory mediators and the immune response. The extent of the inflammatory response after surgery is related to the to the extent of the surgical procedure or tissue injury. The surgical inflammatory response is composed of various components which are hormonal, metabolic, hemodynamic and immune responses. Resolution of the inflammatory response is controlled with anti-inflammatory mediators. Balanced inflammatory reponse vs overwhelmed immune response are the two main reponses which are the main factors in surgical inflammation. The main aim in the treatment is to prevent the overwhelming inflammatory response and reduce organ damage.

Keywords: Surgical inflammation; proinflammatory cytokines; anti-inflammatory cytokines; endothelium; systemic inflammatory response; resolution of the inflammatory response

ÖZET İnflamasyon, çeşitli tehlikeli ve zararlı iç veya dış uyaranlara karşı doku reksiyonudur. Ameliyatın etkisi, cerrahi işlemin yapıldığı yerde lokal olabileceği gibi vücudun uzak bir bölgesinde de olabilir. Cerrahi girişimler farklı etkilerle sistemik inflamasyona yol açabilir: bu etkiler: proinflamatuar sitokinlerin salgılanması, endotel disfonksiyonu, glikokaliks hasarı, nötrofil aktivasyonu ve sonunda doku ve çoklu organ disfonksiyonu. Bu etki inflamatuar mediatörler ve bağışıklık yanıt ile ilişkilidir. Ameliyattan sonra inflamatuar yanıtın boyutu, cerrahi girişimin veya doku hasarının boyutuyla ilişkilidir. Cerrahi inflamatuar yanıt, hormonal, metabolik, hemodinamik ve immün yanıtlar gibi çeşitli bileşenlerden oluşur. İnflamatuar yanıtın çözünürlüğü, antiinflamatuar mediatörler ile kontrol edilir. Dengeli inflamatuar yanıt ve aşırı bağışıklık yanıt, cerrahi inflamasyonda önemli olan iki ana yanıttır. Tedavide temel amaç, aşırı inflamatuar yanıtı önlemek ve organ hasarını azaltmaktır.

Anahtar Kelimeler: Cerrahi inflamasyon; proinflamatuar sitokinler; anti-inflamamatuar sitokinler; endotel; sistemik inflamatuar yanıt sendromu; inflamatuar yanıtın sonlandırılması

The surgical inflammation is considered as a protective response which is composed of overlapping succesive phases surgical inflammation could be either acute or chronic and local or systemic.[1] The aim is to eliminate the cause of and the final necrotic debris which is the result of the cellular injury. There are two scenarios in the process. The good scenario is that the process ends with tissue repair and in the bad scenario the process progresses to systemic inflammatory response and organ damage with failure.

Inflammation is the tissue response to various dangerous and harmful internal or external stimuli. The causes of inlammation are toxic or chemical factors, physical factors such as surgery and trauma, infection, ischemic damage and immune response. The

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effect of surgery could either be local at the site of surgical procedure or at a remote area in the body.[1] Locally this could be related to local tissue injury and loss of the continuity of the skin with a defect in the barrier function and microbial invasion; whereas intravascular canulation, general anesthesia, tracheal intubation or urinary catheterization could be the cause of inlammation in a remote area other than the surgical field. Surgery lead to a tissue injury with an acute inflammatory response secondary to exposure of molecules that are not normally present in the extracellular environment of the organism. This acute inflammatory response results in production of pro-inflammatory cytokines which produces changes in the connective and vascular tissues leading to vasodilatation leading to release of fluid into the extracellular space. This allows the leukocyts and proteins to reach the injury for innate and adaptive mechanisms. Once the stimulus is controlled anti-inflammatory mediators will be released to allow the tissue repair and the normal state is achieved (Figure 1).[2]

Surgical interventions may lead to a systemic inflammation with different effects: secretion of proinflammatory cytokines, endothelial dysfunction, glycocalyx damage, activation of neutrophils, and ultimately tissue and multiple organ dysfunction.[3] Various inflammatory mediators are secreted at a sequential pattern at various levels of the body. With the incision, as a tissue trauma, the first response in the skin leads to secretion of chemokines, kerataocyte chemeoatractant (12 hours), macrophage inflammatory protein (MIP) (24 hours) , activation of macrophages and recruitment of neutrophils. These are followed by secretion of TNF-alpha at the fatty tissue, Damage Associated Molecular Patterns (DAMP's) at the organ level and endothelial cell damage, platelet adhesion and neutrophil influx at the microcirculatory level.[3] Modulation of neutrophil recruitment is essential in limiting the tissue injury and autoimmunity besides targeting the inflammatory cells to limit the effect of invading pathogens at the site of infection.[4]

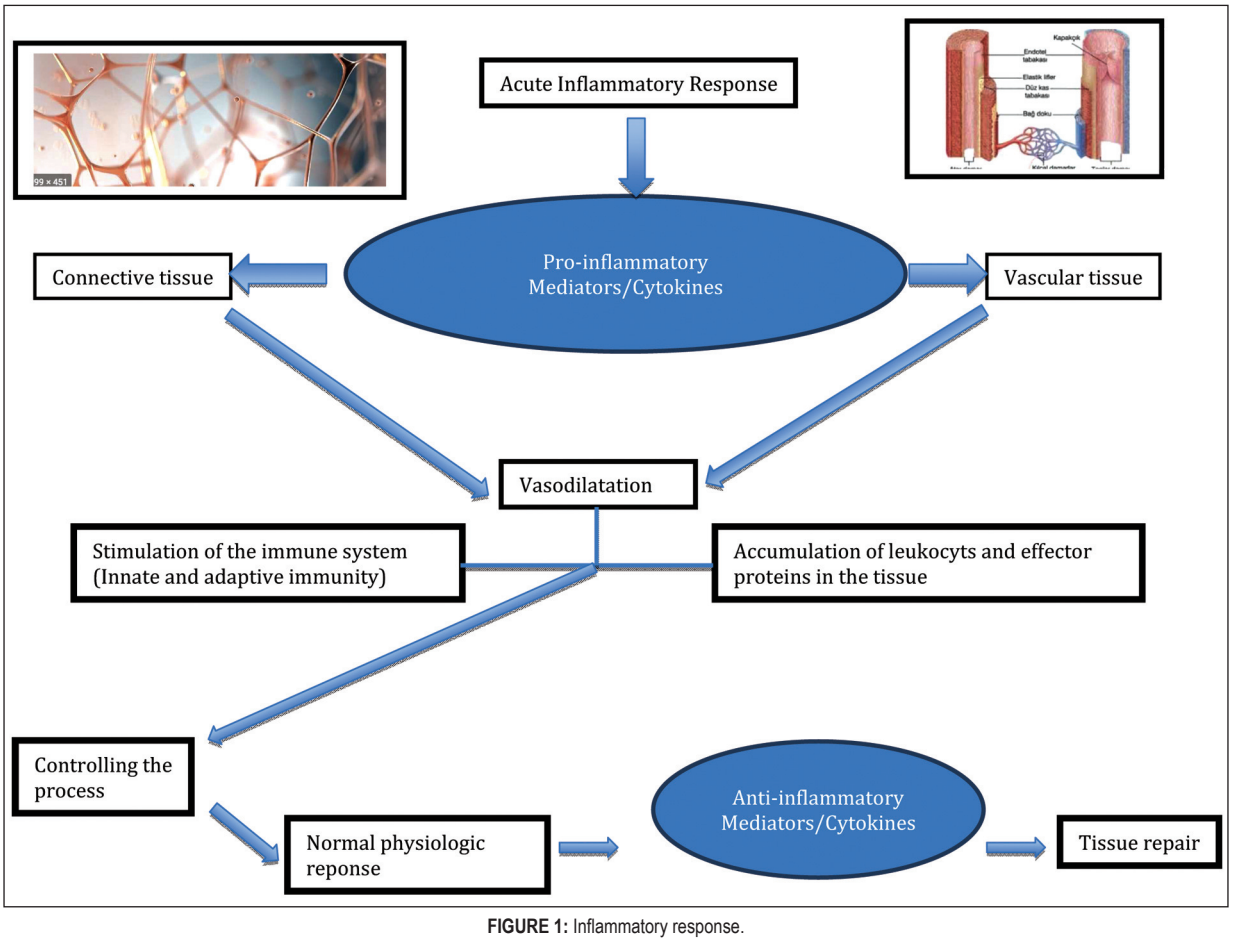


FIGURE 1: Inflammatory response.

Cytokines released after surgical trauma are: TNF-alpha, IL-1Beta, IL-6, IL-10 and IL-2R (>24 hours). The effects of these cytokines, especially if the hemostasis is disregulated, will lead to accumulation of inflammatory cells, neutrophil and macrophage activation, increase in B, T, NK cells and eosinophils. These lead to cellular activation, chemotaxis and microcirculatory disturbances.[3] If the cytokine secretion is overwhelming and uncontrolled, a state known as "cytokine storm" is seen more commonly in ischemia and reperfusion and can lead to cellular death and ultimately to organ damage.

The endothelium is an important target in this process. The endothelial cellular activation and dysfunction is related to three factors: 1) accumulation of inflammatory cells at the injury site, 2) secretion of cytokines and 3) liberation of reactive oxygen species (Oxygen radical, NO i.e). There are two types of endothelial cellular activation. Type 1 is associated with endothelial cell retraction, P-selectin expression and von Willebrand factor secretion leading to increased endothelial permeability. Whereas Type 2 is based on gene expression and denovo protein synthesis including adhesion molecules and tissue factor associated with endothelial cell activation and dysfunction. The endothelium acts as a sensor in systemic inflammation.[3] Various stimuli, such as inflammation and surgery may demonstrate damaging effect on glycocalyx, an endothelial coating, a source of proinflammatory mediators and helps preventing rolling of cells.[3]

The factors determining the effects of inflammation after surgery depends on pro- and anti- inflammatory mediators and the immune response, innate or adaptive.[5] The extent of the inflammatory response after surgery mainly is related to the to the extent of the surgical procedure or tissue injury. Besides these there are other surgical factors as well influencing the perioperative inflammatory response. These factors are: the duration of surgery; presence of infection; changes in leukocytes and platelet count; presence of co-morbidities (atherosclerosis, myocardial infarction, diabetes, ischemic insult etc); smoking; intensity, size and multiplicity of trauma; extracorporeal support, ischemia & reperfusion injury, blood loss and transfusion, high volume ventilation and anesthetic regimen used.[3]

Inflammatory response to surgery is a sequential process which is composed of three phases and has various components. The first phase, neurogenic phase is characterized with oxidative and nitrosative stress; the second phase, immune phase produces an enzymatic stress and third phase, endocrine phase in which an energetic stress is reached. The oxidative stress and edema progressively subsides in the neurogenic phase; the enzymatic stress is followed with neutralization and in the energetic stress the tisuue destruction is followed with tissue repair through regeneration and/or fibroplasia.[1] The surgical inflammatory response is composed of various components which are hormonal, metabolic, hemodynamic and immune responses.[6] The hormonal response is composed of secretion of various stress hormones; the metabolic response is characterized with a hypo metabolic response followed by a hyper-metabolic and catabolic state at day 4-5; and the immunologic response starts with leucocytosis and accumulation of granulocytes and macrophages at the injury site. The immune response is biphasic; at the early stage an immune activation is seen followed by a moderate immune suppression generally after two days.[6]

Bacterial translocation is an important pathway in the development of systemic inflammatory response syndrome. Various substances such as toxins, drugs and pathogens may cause hyper-inflammation which may lead to increased gut permeability (leaky gut) and subsequently bacterial translocation. Leakage of bacteria and metabolites can be cause of acute or chronic inflammation.[7] Bacterial translocation can be seen after elective abdominal surgery, transplant donor surgery, intestinal obstruction, colorectal cancer, ischemia and reperfusion injury, shock, pancreatitis, obstructive jaundice, parenteral nutrition and malnutrition.[8]

Resolution of the inflammatory response is controlled with anti-inflammatory mediators. Regeneration is the best mechanism in resolution as this is a good method of fighting against the energetic stress in the inflammatory response. The cellular mechanisms involved in resolution of inflammatory response preventing infiltration of the polymorphonuclear leukocytes (PMNL) which in turn the vascular permeability and edema would return to normal. The majority of the PMNL's will eliminated from the injury site with apoptosis. After this the monocytes and macrophages would infiltrate the area which helps to eliminate the apoptotic PMNL, microorganisms and the necrotic debris.[9] In recent years it has been reported that some signalling molecules such as lipoxin, resolving, protectin and vasoinhibin are effective in resolution of the inflammatory response.[1]

Balanced inflammatory response vs overwhelmed immune response are the two main responses which are the main factors in surgical inflammation. In a balanced inflammatory response enhances the resistance to infection whereas overwhelmed immune response lead to organ damage and failure. Overwhelming innate immune response or inadequate adaptive immune response are important fac-

tors effecting the morbidity and mortality with increased risk for surgical site infection, systemic inflammatory response syndrome (SIRS) and sepsis.[5]

The main aim in the treatment is to prevent the overwhelming inflammatory response and reduce organ damage. Ischemic and pharmacological priming, optimized positive end expiratory pressure (PEEP 6 cm H20), tocilizumab, steroids, protecting glycocalyx, anti-inflammatory drugs (Ibuprofen), reduce tissue trauma during surgery and removal of cytokines from the medium are treatment methods used.[4] We have shown in an animal study that epinephrine and endotoxin tolerance modulates the cytokine levels and leads to a decrease in the TNF-alfa, IL-6 and IL-10 levels to high dose lipopolysaccharide challenge.[10]

In conclusion; inflammation is a protective response against various stimuli and surgery is one of the causes of inflammation. Inflammation could be acute or chronic and local or systemic. Surgical inflammation is a complex process with hormonal, metabolic, hemodynamic and immunologic interaction. The overwhelming or excessive and uncontrolled reponse may lead to endothelial damage which may either end with resolution and tissue repair or organ damage and failure.

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Idiopathic Granulomatous Mastitis

İdiyopatik Granülomatöz Mastit

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ABSTRACT Idiopathic granulomatous mastitis is a chronic inflammation of parous breast tissue. So far, the etiology, pathophysiology and even optimal treatment have remained unclear. Some immunological alterations, hormonal factors and infectious agents have been suggested to explain etiopathology. However, no single responsible factor has been identified. Histopathology reveals non-caseous granuloma formation in the mammary lobules. Its clinical progression also displays variability from patient to patient. Multiloculated abscess formations and skin fenestrations are common findings in chronic periods. The disease is characterized by chronic self-improvement or self-limitation and recurrence periods. No matter how severe, sepsis and systemic inflammation are not observed during acute attacks. The current treatment strategy for idiopathic granulomatous mastitis is a multimodal approach that includes intermittent observation, cortisone, immunosuppressive agents and various surgical applications.

Keywords: Idiopathic granulomatous mastitis; breast inflammation

ÖZET İdiyopatik granülomatöz mastit, etiyolojisi, patofizyolojisi ve hatta tedavisi bile çok iyi belirlenmemiş, doğurganlık dönemindeki meme dokusuna özgü kronik bir inflamasyondur. İmmünolojik değişimler, bazı anormal hormonal faktörler ve bazı enfeksiyon ajanları etyolojide öne sürülmektedir. Ancak, tek başına sorumlu bir etmen belirlenmiş değildir. Tipik olarak, meme lobüllerinde non-kazeoz granülom formasyonu gözlenir. Klinik progresyonu da tipik olmayan bir şekilde hastadan hastaya oldukça değişkenlik göstermektedir. Multiloküle apse formasyonları ve deriye fenestrasyonları yaygındır. Hastalığın kronik dönemde kendi kendini sınırlama, düzelme ve rekürrens özelliği belirgindir. Akut ataklar sırasında, hastalık ne kadar şiddetli olursa olsun sepsis ve sistemik inflamasyon gözlenmez. İdiyopatik granülomatöz mastitin güncel tedavisi, aralıklı gözlem, kortizon, immün supresif ajanlar ve çeşitli cerrahi uygulamaları içeren multimodal yaklaşımdır.

Anahtar Kelimeler: İdiyopatik granülomatöz mastit; meme inflamasyonu

Idiopathic granulomatous mastitis (IGM) is a rare, -frequently recurring-, chronic inflammation characterized by non-caseous granuloma formation of the breast. The disease was first described as a benign condition that clinically simulated carcinoma by Kessler and Wolloch in 1972.[1] Until now, etiopathogenesis remained unclear. True incidence and prevalence are also unknown due to the relatively high frequency of the disease in certain regions (ie. Asia, Middle-east and Mediterranean countries). The highest number of IGM cases has been published from the Turkish population.[2] Global incidence and prevalence were given as 0,37% and 2,4 per 100.000 respectively.[3,4] IGM commonly affects pareous young women at six months-2 years after cessation of breastfeeding, but it can be observed in lactating breasts (5%) or nulliparous women and very rarely in men.[5]

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CLINICAL FEATURES

In the aspects of heterogeneous inflammatory progression and clinical courses, IGM represents a wide spectrum of clinical entities. The most prevalent clinical sign is a unilate-

rally palpable mass of variable size up to 1-20 cm in diameter.[2,5] In a recently published meta-analysis containing over 3000 patients, mean tumor size and symptom duration were given as 5 cm (3-9 cm, 86% of cases) and 6-7 months (ranging from 1-12 months) respectively.[2] Typically, the masses with obscure margins are quite painful and frequently associated with overlaying skin erythema (Table 1). The inflammatory mass can be seen in any quadrant of the breast, but frequently occur in the retroareolar region. Even with ultrasonography, mammography and breast MR, these clinical findings can sometimes be mistaken for inflammatory breast cancer due to skin edema or non-inflammatory breast cancer based on palpable mass and its radiographic appearance. More rarely, isolated skin changes (without the formation of a mass), skin necrosis and nipple retraction can be seen at presentation as illustrated in Figure 1. Abscesses, sinus formation, skin fenestrations and purulent discharges from the sinuses become noticeable as the clinical picture gets more chronic and mimics tuberculosis and fungal infections ie, actinomycosis at this stage.[5] Due to its heterogeneous clinical presentation and its broad-spectrum differential diagnosis, histopathological confirmation is always mandatory at initial and recurrence episodes. In other words, IGM is diagnosed by histopathology only.[6]

Interestingly, the clinical course of inflammatory mass varies considerably from patient to patient. For example, in some cases, inflammatory mass dampens over time and spontaneously dissolves without any intervention; while in some cases, the illness disseminates through adjacent breast areas by forming new active inflammatory masses, multi-loculated abscesses and multiple skin fenestrations. Even aggressive forms of disease eventually tend to complete healing leaving behind remarkable breast deformity. IGM is an inflammation that is only limited to breast tissue and does not spread to other parts of the body. For example, it does not cause sepsis or systemic inflammation and/or organ failure. However, fever and regional lymphadenopathies can be seen.[2,6] IGM is an inflammation that tends to recur with similar clinical pictures ipsilaterally or contralaterally after full inflammatory resolution. Interestingly, recurrent inflammation almost commonly occurs in previously unaffected regions of the breast (Personal view). The number of recurrence episodes and their intervals and also inflammation intensities in recurrent forms vary from patient to patient. In a published study involving 720 IGM patients from 22 breast centers, pregnancy, breastfeeding, smoking and a history of breast infection were found to be a risk factors for IGM recurrence.[7] But, any type of treatment modality at the initial episode and co-incidental chronic disease were not found to be associated with recurrence.

ETIOLOGY AND PATHOPHYSIOLOGY

Histopathologically, IGM is defined as lobulocentric non-necrotizing granulomatous inflammation, associated commonly with Langhans giant cells, eosinophilic infiltration

TABLE 1: Clinical features of IGM patients in some selected series.

	Çetinkaya et al.[8]	Tan et al.[9]	Uysal et al.[7]
Symptoms & Signs	118 (100 %)	113 (100%)	720 (100%)
Palpable mass	92 (78%)	103 (91%)	587 (83%)
Pain	79 (67%)	76 (67%)	72 (10%)
Fever		4 (3,5%)	
Erythema	50 (42%)	25 (22%)	
Fistula	38 (32%)	5 (4%)	215 (30%)
Lymphadenopathy	63 (57%)		50 (7%)
Parity		112(95%)	96 (85%) 662 (92%)
Breastfeeding	108 (91%)		612 (85%)
Trauma history	117 (92%)		
Smoking	28 (23%)		172 (24%)
Oral contraceptive	18 (15%)		90 (13%)
Comorbid disease			
Diabetes	12 (10%)		14 (2%)
Autoimmun disease			21 (3%)
Hypertention	18 (15%)		29 (4%)



FIGURE 1: Skin necrosis and abscess formation during first recurrence period in a patient with Idiopathic granulomatous mastitis.

and microabscess formation (Figure 2).[10] However, microscopic appearance is not indicative of any kind of specific etiological factor and pathophysiology of the disease.

Three main hypotheses and etiological factors have been postulated to explain IGM pathophysiology: autoimmunity, infectious diseases caused by known and unknown agents and hormonal disturbances.

AUTOIMMUNITY

There is some strong evidence to suggest that IGM has an autoimmune or immunological basis: a) The disease responds quite well to steroid or immuno-suppressive therapy both in initial and recurrence episodes.[11] b) Although IGM is not a systemic autoimmune disease, some systemic granulomatous disorders, in which autoimmunity plays a remarkable role (i.e Crohn's disease, Granulomatosis with polyangiitis (Wegener disease), Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), Sarcoidosis, Ig4-Diseases) can be associated with IGM.[12] c) Extramammary symptoms including arthralgia, erythema nodosum and rheumatoid arthritis are commonly seen in IGM patients. In some patients, high serum Rheumatoid factor (RF), ANA (Anti-nuclear antibody), Anti-DNA antibodies as well as T cell predominance, are suggesting the role of autoimmunity in the pathophysiology of IGM.[4] d) In a recently published study, serum RF, ANA, Anti dsDNA, pANC and Anti-CCP levels were analyzed 3 patient groups (Active IGM patients, patients with IGM history and control patients) and remarkable alterations were found.[13] On the other hand, higher levels of circulating IL-17, IL-22 and IL-23 in active IGM patients as well as CD3, CD4 and CD8 lymphocyte predominances in active lesions indicate cell-mediated immunity in the development of granulomatous mastitis.[14]

INFECTION

Contrary to expectations, routine microbiological cultures commonly reveal no specific infectious agent in abscess/tissue materials.[15] In more specific microbiologic analyses, *Corynebacterium* has been the most linked bacteria for the development of IGM and remarkably, 4 *Corynebacterium* species (*C. kroppenstedtii, C. tuberculostearicum, C. freneyi* and *C. amycolatum*) were isolated from pus or abscess materials in active IGM patients.[4,16] These strains were also isolated from IGM masses successfully.[17] Among the almost 100 *Corynebacterium* species, *C.kroppenstedtii* is the most commonly isolated one by using several detection methods. For example, *C.kroppenstedtii* has been found 33% in granulomatous mastitis and 56% in non-granulomatous lobular mastitis patients using mass spectrometry.[18] The same strain was found in 68% of IGM patients by using real-time PCR and Sanger sequencing on formalin-fixed paraffin-embedded tissue samples.[19] Recently, 2 two novel *Corynebacterium* species quite similar to *C.kroppenstedtii* which potentially cause IGM were found.[20] However, debate about the exact role of *Corynebacteria* on IGM pathogenesis is ongoing due to several reasons: (a) isolated species are mostly non-pathogenic in humans contrary to farm animals and these are the members of normal skin flora. In the published series, inter-patient variability for *Corynebacterium* relative abundance was found to be high from both pus and skin samples amongst patients with IGM. But, *Corynebacterium* relative abundance was found to be similar in both IGM pus and skin samples.[21] Some species including *C. kroppensteadtii* were found to be higher in pus samples than paired skin samples.[21] (b) *Corynebacterium* can be isolated from normal breast tissue.[22] (c) Antibiotics have limited efficacy against IGM progression and recurrence.

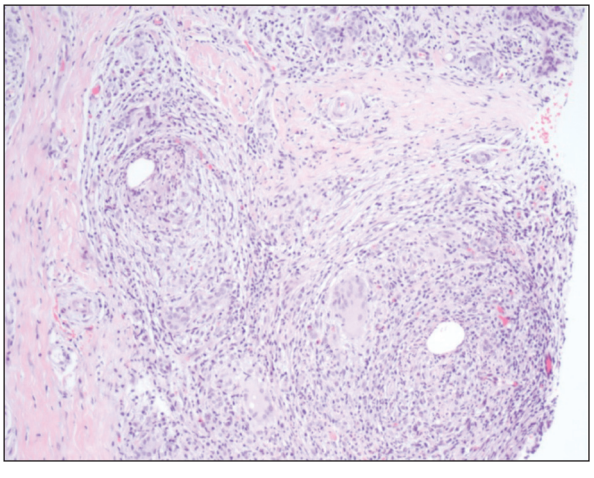


FIGURE 2: Histopathologic appearence in a core biopsy sample *(HE staining, kind contribution of Dr. Necmettin Özdemir)*

Besides *Corynebacterium* species, several microorganisms including *Staphylococcus, Acinetobacteria, Actinomicosis, Blastomycosis* have been cultured from IGM materials.[4] However, no IGM-specific dominant genus has been observed in detailed microbiologic analyses.[21]

HORMONAL FACTORS

The hormonal disorder hypothesis in IGM pathogenesis stemmed from the evidence of elevated serum prolactin levels in some IGM patients. However, IGM is remarkably rare during pregnancy when serum prolactin levels are physiologically high. Also, constantly elevated prolactin levels have not been confirmed in large case series.

Oral contraceptive usage was considered as another causative hormonal factor for IGM pathogenesis and the frequency of IGM patients taking oral contraceptives was given between 11% and 42% in the published series.[23]

TREATMENT

There is no standard therapy or consensus for the treatment of IGM yet due to its obscure etiology, diverse clinical scenarios and its self-limiting feature. Treatment options range from observation without any medication, non-operative treatments including antibiotics, steroids, immuno-suppressive and anti-inflammatory agents to operative treatments including surgical drainage, wide excision and partial mastectomy. The commonly accepted treatment strategy is a multimodal approach, -depending on the clinical courses of IGM-, by using treatment alternatives sequentially or combining each other until full resolution at first or recurrence periods. The "watch and wait" option seems to be potentially effective for all IGM conditions considering the long recovery time. Çetinkaya et al. compared the observation (42% of 118 pts) with systemic steroid treatment prospectively.[8] They found mean recovery times of 3,9 and 5,6 months in observation and steroid groups, respectively. However, complete resolution commonly takes 9-12 months under observation.[24] In routine practice, the "no-treatment option" has been preferred for localized disease and small lesions.[25]

Although, antibiotics have been using almost all patients as first-line treatment, but therapeutic efficacy of the antibiotics is quite low. Because IGM-related abscesses are mostly sterile and isolated microorganisms are mostly members of normal skin flora. In addition, the role of microorganisms in IGM etiology remained unclear. According to Achajanzadeh et al. treatment with only antibiotics reduced clinical symptoms and signs in only 3% of patients.[26] But, 97% of the women who failed to respond to antibiotics (200 pts) were treated with systemic steroids and finally, clinical relief was obtained in 72% of patients. On the other hand, *Corynebacterium*-targeted antimicrobial therapies have been found ineffective against the clinical progression of IGM.[17] Among the non-operative options, steroids seems to be most effective treatment. Moreover, steroid treatment is not differ from surgery in terms of time-to-healing and recurrence of disease.[27] In addition, no difference has been found between systemic and local steroid administrations.[28]

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Inflammation and Organ Transplantation

İnflamasyon ve Organ Transplantasyonu

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ABSTRACT Oxidative stress is a major and recurrent cause of inflammation in organ transplantation. The ischemia-reperfusion damage that occurs during organ transplantations causes emergence of free oxygen radicals and pathological nitrogen products. This increases oxidative stress. Reducing oxidative stress leads to increased consumption of endogenous antioxidants. Depending on the degree of oxidative stress, various degrees of damage may occur in the transplanted organ, and as a result, the function of the graft may be impaired. The decrease in adenosine triphosphate (ATP) level formed during ischemia leads to acidification in the intracellular and extracellular environment. This results in accumulation of sodium and calcium in the intracellular environment. Calcium ion accumulation leads to activation of calcium-dependent proteases, which initiates irreversible cell membrane damage that causes necrosis, apoptosis, and autophagic mechanisms. Especially increase in superoxide radicals and proinflammatory factors, decrease in nitric oxide production and activation of hypoxatine-xanthine oxidase system in the liver increase hepatocellular damage by increasing oxidative stress. While ischemia creates serious tissue damage, reperfusion of the organ can lead to more severe damage. The increase in oxygen levels with reperfusion may contribute to the increase of reactive oxygen products. It has been shown that the main cells that initiate ischemia-reperfusion injury in the liver are Kupfer Cells, and the main mediators are reactive oxygen and reactive nitrogen products. Reactive oxygen products include hydrogen peroxide (H2O2), superoxide anion and hydroxyl radicals. The most biologically relevant reactive nitrogen product is nitric oxide (NO). Activation of Kupfer Cells together with reactive oxygen products causes formation of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). DNA damage and endothelial dysfunction may also occur during ischemia-reperfusion injury. There is no proven treatment to prevent ischemia-reperfusion injury during reperfusion, other than ischemic preconditioning. During reperfusion, Kupfer Cells stimulate the secretion of CD4+ T lymphocytes; proinflammatory cytokines such as TNF-alpha and IL-1 are activated. Anti-inflammatory cytokines such as IL-4 and IL-10 also work to reduce ischemia-reperfusion injury. Meanwhile, there is a decrease in the levels of many antioxidants. In the late phase of reperfusion, reactive oxygen products and proinflammatory mediators can activate sinusoidal endothelial and CD4+ T cells, and they attract neutrophils to the injury site. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules (VCM-1) secreted by endothelial cells increase neutrophil infiltration. Activation of innate immune responses is observed during ischemia-reperfusion injury. As a result, reactive oxygen products from damaged cells and damage associated molecular patterns (DAMP) such as high mobility group box-1 (HMGB-1) and heat shock proteins (HSPs) emerge. These are recognized by Toll-like receptors (TLRs). Activation of signal transduction proteins and transcription factors by TLRs results in the production and release of inflammatory cytokines and chemokines that enhance dendritic cell maturation. N-acetyl cysteine is beneficial in many stages in reducing ischemia-reperfusion injury. In addition, to reduce inflammation in the graft; antiischemic interventions, therapies targeting TLRs and Nuclear factor KappaB, therapies that reduce inflammatory cytokines and adhesion molecules, complement inhibition and antiapoptotic strategies can be used. Transplanted tissues can be rejected as hyperacute, acute and chronic. The most important mechanism in the prevention of rejections is immunosuppression treatments that prevent the activation and functions of T cells.

Keywords: Inflammation; organ transplantation; ischemia-reperfusion injury

ÖZET Organ transplantasyonunda oksidatif stres, inflamasyona neden olan majör ve tekrarlayan bir nedendir. Organ transplantasyonları sırasında oluşan iskemi-reperfüzyon hasarı serbest oksijen radikalleri ve patolojik nitrojen ürünlerinin ortaya çıkmasına neden olur ve bu durum oksidatif stresi arttırır. Oksidatif stresi azaltmak için endojen antioksidan tüketiminde artma olur. Oluşan oksidatif stresin derecesiyle orantılı olarak transplante edilen organda değişik derecelerde hasar oluşabilir ve bunun sonucu olarak graftın işlevlerinde bozulma görülebilir. İskemi sırasında oluşan adenozin trifosfat (ATP) düzeyinde azalma intrasellüler ve ekstrasellüler ortamda asidifikasyona yol açar. Bu durumda intrasellüler ortamda sodyum ve kalsiyum birikimine neden olur. Kalsiyum iyonu birikimi, kalsiyum bağımlı proteazların aktivasyonuna yol açarak nekroz, apoptoz ve otofajik mekanizmaların oluşmasına neden olan geri dönüşümü olmayan hücre membran hasarını başlatır. Özellikle karaciğerde süperoksit radikalleri ve proinflamatuvar faktörlerde artma, nitrik oksit üretiminde azalma ve hipoksatin-ksantin oksidaz sisteminde aktivasyon oksidatif stresi yükselterek hepatosellüler hasarı arttırır. İskemi ciddi bir doku hasarı oluştururken, organın reperfüzyonu daha şiddetli bir hasarlanmaya yol açabilir. Reperfüzyonla birlikte oksijen düzeylerindeki artma reaktif oksijen ürünlerinin artmasına katkı sağlayabilir. Karaciğerde iskemi reperüzyon hasarını başlatan ana hücrelerin Kuppfer Hücreleri, ana mediatörlerin de reaktif oksijen ve reaktif nitrojen ürünleri olduğu gösterilmiştir. Reaktif oksijen ürünleri; hidrojen peroksit(H2O2), süperoksit anyonu ve hidroksil radikallerini içerir. Biyolojik olarak en alakalı reaktif nitrojen ürünü ise nitrik oksittir (NO). Reaktif oksijen ürünleri ile birlikte Kuppfer Hücreleri'nin aktivasyonu, tümör nekroz faktör-alfa (TNF-alfa) ve interlökin-1 (IL-1) oluşmasına neden olur. İskemi-reperfüzyon hasarı sırasında DNA hasarı ve endotel disfonksiyonu da ortaya çıkabilir. Reperfüzyon sırasında, iskemik ön koşullandırma dışında iskemi-reperfüzyon hasarını önlemek için etkinliği kanıtlanmış bir tedavi bulunmamaktadır. Reperfüzyon sırasında Kuppfer Hücreleri tarafından aktive edilen CD4+ T lenfositlerin salgılanmasını uyardığı TNF-alfa ve IL-1 gibi proinflamatuvar sitokinler aktive olurken; IL-4 ve IL-10 gibi antiinflamatuvar sitokinler de iskemi-reperfüzyon hasarının azaltılmasını sağlamaya çalışır. Bu sırada birçok antioksidan düzeyinde azalma görülür. Reperfüzyonun geç fazında reaktif oksijen ürünleri ve proinflamatuar mediatörler, sinüzoidal endotel ve CD4+ T hücreleri hücrelerini aktive edebilir ve nötrofillerin hasar alanına çekilmesine neden olur. Endotel hücreleri tarafından salgılanan intersellüler adezyon molekül-1(ICAM-1) ve vasküler hücre adezyon molekülleri (VCM-1) nötrofil infiltrasyonunu arttırır. İskemi-reperfüzyon hasarı sırasında doğuştan gelen (innate) immün yanıtlarda aktivasyon görülür. Bunun sonucunda hasarlı hücrelerden reaktif oksijen ürünleri ve sonrasında yüksek mobilite grubu box-1 (HMGB-1) ve ısı şoku proteinler (HSP'ler) gibi damage associated molecular patterns (DAMP) ortaya çıkar. Bunlar Toll-like reseptörler (TLR'ler) tarafından tanınır. TLR'ler tarafından sinyal transdüksiyon proteinlerini ve transkripsiyon faktörlerini aktive edilmesi, dendritik hücre olgunlaşmasını güçlendiren inflamatuar sitokinlerin ve kemokinlerin üretimi ve salınımı ile sonuçlanır. İskemi reperfüzyon hasarını azaltmada N-asetil sistein birçok aşamada yararlı olmaktadır. Ayrıca grafttaki inflamasyonu azaltmak için; antiiskemik girişimler, TLR'leri ve Nükleer faktör KappaB'yi hedefleyen tedaviler, inflamatuvar sitokin ve adhezyon moleküllerini azaltmayı sağlayan tedaviler, kompleman inhibisyonu ve antiapoptotik stratetejiler kullanılabilir. Transplante dokular, hiperakut, akut ve kronik olarak reddedilebilmektedir. Rejeksiyonların önlenmesinde en önemli mekanizma T hücre aktivasyon ve işlevlerinin engellenmesini sağlayan immünsupresyon tedavileridir.

Anahtar Kelimeler: İnflamasyon; organ transplantasyonu; iskemi-reperfüzyon hasarı

Oxidative stress is a major and recurrent cause of inflammation in organ transplantation. Inflammation after organ transplantation can occur after ischemia and reperfusion injury, organ rejection and graft infections caused by different microorganisms (*Cytomegalovirus*, etc.) after transplantation.

ISCHEMIA AND REPERFUSION INJURY

Ischemia-reperfusion injury during organ transplantation causes inflammatory cell migration to the site of injury. Free oxygen radicals and pathologic nitrogen products from these cells increase oxidative stress. Endogenous antioxidant consumption increases to reduce oxidative stress. In proportion to the degree of oxidative stress, different degrees of damage may occur in the transplanted organ and as a result, impairment in the function of the graft may occur.[1]

During organ transplantation, cellular stress can occur throughout the entire process. In fact, cellular stress starts in the donor body before the transplantation of the organ to the patient. Many hemodynamic and neuroendocrine changes occur in the donor with brain death, multiorgan harvesting, cold and hot ischemia. Later, hemodynamic changes, warm ischemia and reperfusion during transplantation of the organ to the recipient also contribute to cellular damage and inflammation. During this process, damage associated molecular pattern (DAMP) formation increases.[1]

The decrease in adenosine triphosphate (ATP) levels during ischemia during and after liver transplantation leads to acidification in the intracellular and extracellular environment. This leads to accumulation of sodium and calcium in the intracellular environment. Calcium ion accumulation leads to activation of calcium-dependent proteases and initiates irreversible cell membrane damage leading to necrosis, apoptosis and autophagic mechanisms. Especially in the liver, an increase in superoxide radicals and proinflammatory factors, a decrease in nitric oxide production and activation of the hypoxanthine-xanthine oxidase system increase hepatocellular damage by increasing oxidative stress. While ischemia causes severe tissue damage, reperfusion of the organ can lead to more severe damage. The increase in oxygen levels with reperfusion may contribute to an increase in reactive oxygen species. Studies have shown that the main cells initiating ischemia-reperfusion injury in the liver are Kuppfer cells and the main mediators are reactive oxygen and reactive nitrogen species. Reactive oxygen species include hydrogen peroxide (H2O2), superoxide anion and hydroxyl radicals. The most biologically relevant reactive nitrogen species is nitric oxide (NO). Activation of Kuppfer cells with reactive oxygen species leads to the formation of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). DNA damage and endothelial dysfunction also occur during ischemia-reperfusion injury.[1]

During reperfusion, there is no proven treatment to prevent ischemia-reperfusion injury except ischemic preconditioning. During reperfusion, Kuppfer cells stimulate the secretion of CD4+ T lymphocytes. Proinflammatory cytokines such as TNF-alpha and IL-1 are activated. Anti-inflammatory cytokines such as IL-4 and IL-10 try to reduce ischemia-reperfusion injury. In the meantime, many antioxidant levels decrease. In the late phase of reperfusion, reactive oxygen species and proinflammatory mediators can activate sinusoidal endothelial and CD4+ T cells, which in turn attract neutrophils to the site of injury. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules (VCM-1) secreted by endothelial cells increase neutrophil infiltration.[1,2]

During ischemia-reperfusion injury, innate immune responses are activated. As a result, reactive oxygen species and subsequently damage associated molecular patterns (DAMPs) such as high mobility group box-1 (HMGB-1) and heat shock proteins (HSPs) are released from damaged cells. These are recognized by Toll-like receptors (TLRs). Activation of signal transduction proteins and transcription factors by TLRs results in the production and release of inflammatory cytokines and chemokines that enhance dendritic cell maturation.

TREATMENTS AIMED TO REDUCE INFLAMMATION AFTER TRANSPLANTATION

N-acetyl cysteine is useful at many stages in reducing ischemia reperfusion injury. Additionally, anti-ischemic interventions, treatments targeting TLRs and Nuclear Factor KappaB, treatments that reduce inflammatory cytokines and adhesion molecules, complement inhibition and anti-apoptotic strategies can be used to reduce inflammation in the graft.

The use of free radical scavengers significantly improves transplantation outcomes.[3] Hypoxia-Inducible Factor-1 Alpha (HIF-1alpha) has a key role in the regulation of different genes in cell adaptation to hypoxia. Heme oxygenase-1 (HO-1) downregulates HIF-1alpha, providing anti-apoptotic effect and reducing tissue I/R injury. Transfection of rat liver allografts with HO-1 before transplantation improves recipient outcomes.

Trimetazidine prevents ATP depletion, lipid peroxidation and acidosis in ITS after hypoxia. Autotransplanted porcine kidneys have been shown to increase graft function and survival when added to cold storage fluids.[4] Polyethylene glycol perfusion may have an effect by reducing graft damage after small intestine, heart, liver and kidney transplantation.[5] In cases where pulmonary catalase is immune-targeted before transplantation in rats, a decrease in ischemia reperfusion injury and an increase in lung transplant functions can be achieved.[6] Flushing liver grafts with tacrolimus may promote ROS detoxification by reducing the rate of oxidized glutathione in humans and mice.[7]

An increase in TLR 4 expression was observed after renal ischemia in mice.[8] Reducing TLR 4 may lead to a decrease in kidney damage and inflammation after ischemia-reperfusion and an improvement in graft functions. Moreover; in cases where there is loss of function in the TLR 4 allele, increased function in the graft can be achieved by increasing hemoxygenase 1 in the graft.[9]

NF-κB is an important down-stream transcription factor in the TLR pathway. It is responsible for the activation of numerous genes involved in the production of inflammatory cytokines, adhesion molecules and chemokines. Decreased NF-κB levels are associated with decreased ischemia-reperfusion injury, increased allograft survival, and decreased IL-1 and TNF-alpha in the graft.[10] NF-κB inhibition in renal allografts leads to a decrease in the intracellular adhesion molecule vascular cell adhesion molecule-1 and monocyte chemotactic protein-1, thereby increasing graft survival.

Interleukin-6 (IL-6) is one of the cytokines that play a key role in ischemia-reperfusion injury. Treatments that reduce interleukin-6 may decrease the amount of effector T cells and increase graft survival. Research on this subject is ongoing.[11] Tumor necrosis factor alpha (TNF-alpha) expression, which has an important role in ischemia-reperfusion injury, can be reduced by short-hairpin RNA (shRNA).

Activation of complement proteins increases after brain death.[12] As known, membrane attack complex (MAC) formation, endothelial cell activation and cytokine production increase after complement activation. Zheng et al. reported that desensitization of C3 and C5a receptors using interfering RNA reduced ischemia-reperfusion injury in mouse kidney.[13] Eculizumab is a monoclonal antibody to C5 that reduces C5 cleavage and thereby MAC formation.

Anti-apoptotic therapies may also contribute to survival in transplanted grafts. Activated T cells and natural killer (NK) cells express Fas ligand (FasL), which induces cell death in Fas-bearing cells. Therefore, it is hypothesized that graft survival can be improved by suppressing the Fas gene or inducing FasL in the graft. In studies, co-transfection of rat liver allografts containing FasL and bcl-2 (anti-apoptotic gene) was observed to increase graft survival.[14] In addition, caspase 3 is a major enzyme in the apoptosis cascade and its gene expression is increased in ischemia-reperfusion injury. Molecules involved in apoptosis such as caspase 3 and 8 are the targets of siRNA therapy to reduce I/R injury.

Transplanted tissues can be rejected as hyperacute, acute or chronic. The most important mechanism in preventing rejection is immunosuppression treatments that prevent T cell activation and functions.

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Herbal Medicines with Anti-inflammatory Effect

Bitkisel Anti-inflamatuarlar

Erdem YEŞİLADA *(1949-2023)*

ABSTRACT Scientific evidence has revealed that oxidation and inflammation are the main triggering factors inducing a wide range of diseases in the body. A range of anti-inflammatory agents exist to help control inflammation; however, they often have side effects and may sometimes not be sufficiently effective. On the other hand, several phytochemicals in herbals have been clinically proven to exert anti-inflammatory activity without severe health risks. Among these, curcuminoids from turmeric rhizomes, gingerols from ginger rhizomes, and salicin from willow barks are examples of phenolics in anti-inflammatory herbals. Moreover, several triterpenes, including boswellic acids from Boswellia oleoresin, asiaticosides and madecassosides from Centella leaves, were also reported as an anti-inflammatory. In addition, lipid-type components, i.e. galactolipids from rosehips, iridoid-type components, i.e. harpagosides from devil's claw roots, and oleuropein in olive leaves and bromelain enzyme from pineapple stems are recognized with significant anti-inflammatory activity. The efficiency of these well-known herbals will be discussed.

Keywords: Inflammation; phytotherapy; triterpenes; iridoids; flavonoids

ÖZET Bilimsel araştırmalar oksidasyon ve enflamasyonun vücutta hastalıkların oluşumunda önemli bir tetikleyici etken olduğunu ortaya koymaktadır. Mevcut anti-inflamatuar ajanlar inflamatuar tepkimelerin kontrolünde yardımcı olmaktadır; ancak genellikle yan etkileri ve bazı durumlarda etkinlikleri tartışılmaktadır. Diğer taraftan, bitkilerin bileşiminde bulunan bazı fitokimyasal ajanların, ciddi olumsuz etkilere yol açmadan anti-inflamatuar etki gösterdiği klinik olarak kanıtlanmıştır. Bunlar arasında zerdeçal rizomlarında bulunan kurkuminoitler, zencefil rizomlarında bulunan gingeroller ve söğüt kabuklarında bulunan salisin anti-inflamatuar etkili fenolik bileşenlere örnektir. Ayrıca triterpenler arasında akgünlük reçinesinde bulunan boswelik asitler, Sentella yapraklarından asiatikozit ve madekassozit'in anti-inflamatuvar etkileri gösterilmiştir. Bunların haricinde, lipit yapısında bileşenler; mesela kuşburnu meyvelerinin galaktolipit fraksiyonu; ananas gövdelerinde bulunan bir enzim olan bromelin ve iridoid tipi bileşenler arasında ise şeytan pençesi rizomlarından harpagozitler ve zeytin yaprağında bulunan oleuropein'in belirgin antiinflamatuar etkisi bulunduğu bildirilmektedir. Bu çalışmada bitkisel kaynaklı anti-inflamatuarların etkinliği konusunda mevcut bazı bilimsel kanıtlar kısaca tartışılacaktır.

Anahtar Kelimeler: Yangı; fitoterapi; triterpenler; iridoidler; flavonoitler

Inflammation is characterized by swelling, redness, pain, and increased body temperature triggered by harmful foreign stimuli, i.e., pathogens, toxic agents, allergens, and mechanical and thermal factors leading to loss of tissue functions. Consequently, the host immune system initiates the healing process by modulating the physiological processes.[1] Inflammatory disorders are a major global health issue, causing an increased morbidity and mortality rate every year. Anti-inflammatory drugs (NSAIDs) are prescribed to manage inflammation and pain when conditions become severe. However, chemical NSAIDs are frequently associated with adverse effects on gas-

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TABLE 1: Well-known herbals used in various inflammatory problems.

Antiinflammatory effect: Inhibits inflammatory pathway
• Ginger (*Zingiber officinale*), rhizome
• Turmeric (*Curcuma longa*), rhizome
• Bromelain (*Ananas comosus*), enzyme
Analgesic effect: Inhibits pain
• Devil's claw (*Harpagophytum procumbens*), root
• Willow (*Salix alba*), bark
Bone regeneration, to inhibit bone resorption
• Indian olibanum (*Boswellia serrata*), oleoresin
• Gotu kola (*Centella asiatica*), leaf
• Dog rose (*Rosa canina*), fruit
• Olive (*Olea Europea*), leaf

trointestinal, cardiovascular, nervous, hepatic, respiratory, renal, and hematologic systems.[2] Therefore, there is a need for safer alternative anti-inflammatory drugs.

Scientific experimentation has shown that many phytochemicals, including polyphenolics, triterpenes, monoterpenes, lignans, etc., exert anti-inflammatory activity. However, if clinically proven evidence of their efficiency is considered, only a small number of them have found a place in medical practice (Table 1).

INDIAN OLIBANUM, FRANKINCENSE (*BOSWELLIA SERRATA*), OLEORESIN

This is an oleoresin obtained from the stems of *Boswellia serrata* by injury. The plant is native to India and surrounding countries, and the pentacyclic triterpene acids named ß-boswellic acids (11-keto-; acetyl- and acetyl-11-keto- derivatives) are defined as the primary active components in the oleoresin. Boswellic acids have been reported to inhibit leukotrienes by interfering with the 5-lipoxygenase pathway and reducing human leukocyte elastase enzyme activity. Its inhibitory activity on matrix metalloproteinases provides reduced destruction in glycosaminoglycans. Randomized Controlled Trial (RCT) studies have confirmed that *Boswellia serrata* extract acts as an antiarthritic, anti-inflammatory, and analgesic and regulates joint functions. Therefore it has become prescribed for osteoarthritis and other arthritic patients for symptom relief and restoration of articular cartilage damage.[3,4] After clinical confirmation of these efficacies, the USA's annual demand for *Boswellia serrata* oleoresin has bounded from $140,000 (in 2013) to $14,600,000 (in 2017). To supply such an increased demand, the resins of other *Boswellia* species (*B. carterii, B. frereana*, *B. papyrifera* and *B. sacra*) from Middle-east and North African sources have become available in the market, despite not being clinically investigated.[5] Therefore, the main source of the extract in the marketing formulations should be questioned.

GINGER (*ZINGIBER OFFICINALE* ROSCOE), RHIZOME

Ginger rhizome has long been used in the world as a popular spice food and medicinal herb because of its high content of antioxidant and anti-inflammatory properties. Ginger rhizome comprises various kinds of chemicals, including 6- and 8-series of gingerols, and shogaols, among which gingerol is the primary ingredient representing a variety of bioactivities in the fresh rhizomes, while converted into shogaol in the dried rhizomes by H_2O loss. Studies have shown that 6-gingerol inhibited the TNF-α, and IL-1β-induced increase in the p38-dependent NF-κB activation and expression of pro-inflammatory genes of IL-6 and IL-8 in normal prostatic epithelial cells.[6] Compressive studies in recent years have displayed that 6-gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-κB in phorbol ester-stimulated mouse skin.[7] 6-Shogaol suppressed LPS-induced up-expression of iNOS and COX-2 in murine macrophages.[8] These effects may be due to the pharmacological activity of biologically active compounds in reducing inflammation.[9]

RCTs demonstrated a significant impact of ginger in lowering circulating CRP, hs-CRP, IL-6 and TNF-α levels.[10] Ginger is well-tolerated, with a low side effect profile, mainly gastrointestinal complaints in those sensitive people. Eventually, Ginger has been suggested as a safe remedy in osteoarthritis patients to reduce pain and motility complaints.[11] Among the attempts to search for higher efficacy, a combination of standardized Boswellia extract and ginger extract has been reported to act synergistically in osteoarthritis cases. The formulation exerted significant joint improvement to slow the degenerative stage by reducing macrophages, monocytes and leucocytes migration through LOX enzyme inhibition and initiating the repairment stage.[12]

TURMERIC (*CURCUMA LONGA*), RHIZOMES

Curcuminoids are the bioactive polyphenols from the underground rhizomes of turmeric with a potent anti-inflam-

matory activity that is safe even at doses as high as 8 g/day for three months or 12 g as a single dose. Clinical evidence suggests it lowers markers of systemic inflammation in humans; however, the optimal dose, formulation, and duration remain inconclusive. Curcumin is metabolized rapidly in the liver for elimination in the urine and feces. However, for curcumin to act on the biological targets of systemic inflammation, including nuclear factor KB (NF-kB) and 5-lipoxygenase for a reduction in pro-inflammatory mediators, its bioavailability is an important consideration for the effects of inflammatory disease. Therefore average absorption rate of curcumin is about 1%, which is too tiny for any therapeutical claim. Among the commonly practised attempts by laypeople to increase the absorption rate is the addition of 1% black pepper (piperine); the rate only reaches 2%. For this reason, pharmaceutic formulations in a lipophilic matrix, such as phytosome (Meriva®) and liposome, successively provide 29 times and 20 times higher absorption rates.[13] Solid-lipid nanoparticles (Theracurmin®, Gelucire®), bio-optimized curcumin (Flexifytol®, Biocure®) and polymeric micelles, etc., are among the other marketing pharmaceutical formulations with higher absorption claims.[14]

Curcumin supplementation resulted in a significant reduction in CRP, IL-6, IL-8, and MCP-1 concentrations and increased anti-inflammatory cytokine IL-10.[15] Clinical studies have confirmed that curcumin may act with a similar potency to NSAIDs (ibuprofen) or potentiate their efficacy (diclofenac) in osteoarthritis patients, without any noteworthy side effects.[16-18] On the other hand, a combination of turmeric extract with *Boswellia serrata* extract significantly reduced pain in osteoarthritis patients.[19]

BROMELAIN

Bromelain is a proteolytic enzyme mainly obtained from the stems of pineapple (*Ananas comosus*) and is also found in fruits with a lower concentration. Currently, bromelain is administered for numerous clinical applications due to its therapeutic effects in treating inflammation and soft tissue injuries. Bromelain exerted anti-inflammatory activity in 240 mg for five days in clinical practice, which helped to inhibit edema and related pain in osteoarthritis and other arthritis cases.[20,21] Also been reported to increase the absorption of glucosamine and chondroitin sulphates, prescribed in the treatment protocols for osteoarthritis. A clinical study demonstrated that bromelain was administered to patients with arthritic joint swelling, and a significant decrease in soft tissue swelling was observed.[22] A meta-analysis study also showed that bromelain was found effective in controlling pain, edema, and trismus after lower third molar surgeries.[23] A clinical study demonstrated that bromelain administered to boxers completely cleared all bruises on the face and haematomas of the orbits, lips, ears, chest and arms within four days.[24]

Numerous reports have documented the benefits of bromelain for also sinusitis.[25,26] A previous study showed that the administration of bromelain among children suffering from acute sinusitis shortened the duration of symptoms and accelerated recovery compared to usual care regimens.[27] Furthermore, sinusitis patients that received bromelain demonstrated complete resolution of breathing difficulties and inflammation of the nasal mucosa.[28]

DEVIL'S CLAW (*HARPAGOPHYTUM PROCUMBENS*), ROOT

Iridoids (harpagoside, harpagid) are the main active anti-inflammatory components in the roots of the devil's claw. They are particularly used in osteoarthritis and lumbago with high tolerability.[29,30] Therefore, it is suggested for individuals sensitive to NSAIDs or gastritis patients. When used in 30-100 mg harpagoside equivalent doses, the activity starts only after two weeks of sustained use and reaches the maximum level within 3-4 months.[31,32] Harpagoside is sensitive to gastric acidity and hydrolyzed to inactive aglycone harpagogenin. Therefore encapsulated formulations for direct delivery to the intestines are required for higher efficacy, or the formulations should be orally taken 2 hrs before or after meals. Meta-analysis studies have shown that THE standardized extracts of devil's claw are effective in osteoarthritis patients, with a similar potency to those of NSAIDs such as diacerein, diclofenac (Voltaren®) and rofecoxib (Vioxx®), but exert higher tolerability.[33-35]

WILLOW (*SALIX ALBA*), BARK

Salicin and other phenolic glycosides (salicortin, tremulin, tremulasin) from the barks of the stem or branches of *Salix* species (*S. alba, S. purpura, S. fragilis*) are recognized as the source of salicylic acid which was then derivatized to acetylsalicylic acids, a well-known pain killer, Aspirin®. However, the activity profiles of acetylsalicylic acid and salicin derivatives are not similar. Due to the irritating nature of acetylsalicylic acid on the gastrointestinal system, its use in sensitive individuals is limited. Also, it acts as a potent antiplatelet. On the other hand, salicin derivatives

do not induce irritation in the gastrointestinal mucosa since they are oxidized to the active metabolite salicylic acid in the liver. Since this conversion occurs in the intestinal lumen, gradually, salicin activity starts late but lasts up to 8 hours. Moreover, as opposed to acetylsalicylic acid, the antiplatelet effect is transient; in other words, it would not increase the efficiency of antiplatelet drugs. However may be contraindicated in individuals sensitive to salicylates and patients with asthma, gut, peptic ulcer, liver and kidney. The daily suggested dose is between 60-240 mg salicin equivalent.[36]

Clinical studies demonstrated that its standardized ethanolic extracts are beneficial for patients with chronic low back pain and rheumatic pain depending on the dose and duration of treatment between 20-40% inhibition in pain as single or combined formulations.[37-40]

OLIVE (*OLEA EUROPEA*), LEAF

In olive leaves, phenolics (oleuropein, tyrosol, hydroxytyrosol) have been reported as active anti-inflammatory components. Animal experiments revealed that a 100 mg/kg dose for eight weeks to mice or at 500 mg/kg for three weeks in rabbits restored the cartilage damage by increasing the synthesis of high molecular weight hyaluronan. These results were also confirmed by a multicentered and randomised clinical study carried out in 125 mg x2 times standardized olive leaf extract administration for 6 months in 124 patients suffering pain in motion.[41-43]

DOG ROSE (*ROSA CANINA*), FRUIT

The fruit of the dog rose, Rosehips, is composed of two parts. The outside yellow to red colored hypanthium is rich in vitamin C, serving as a cofactor of the enzyme responsible for collagen synthesis and tissue repair, while the achene fruits inside contain galactolipid fraction with anti-inflammatory activity. The powdered fruits or extracts have been clinically shown to inhibit pain and joint stiffness, increase flexibility and reduce the need for pharmacotherapy in osteoarthritis and rheumatic arthritis patients.

A meta-analysis of three randomised controlled trials involving 287 patients for an average three months treatment period with standardised rosehip powder consistently reduced pain scores. It showed that patients allocated to rosehip powder were twice as likely to respond to rosehip compared to placebo. In contrast to NSAIDs, rosehip treatment did not induce any gastric damage or did not affect the coagulation cascade or fibrinolysis.[44,45]

GOTU KOLA, CENTELLA (*CENTELLA ASIATICA*), LEAF

A mixture of pentacyclic triterpene saponins, *i.e*., asiaticosides (40%), madecassic acid (30%) and asiatic acid (30%) is called "Total Triterpene Fraction of *Centella asiatica*" (TTFCA). This mixture acts as an antioxidant and anti-inflammatory. Scientific research has revealed that TTFCA increases collagen type 1 synthesis, myofibroblast production, and angiogenesis. Therefore has been used for the rapid regeneration of joints orally and externally in dermatological and cosmeceutical formulations for wound healing and as anti-aging.[46]

CONCLUSION

Oxidative stress triggers the inflammatory pathway leading to acute and chronic inflammatory diseases. Scientific evidence has shown that inflammation takes an active role in the pathogenesis of almost all diseases. A common approach in current medical practice is prescribing NSAID agents for treating such disorders. However, there are limitations in use due to their severe gastrointestinal damage and bleeding risks. However, phytochemicals with anti-inflammatory activity are distributed widely in the plant kingdom. A large body of experimental evidence has accumulated that particularly phenolics (phenolic acids, flavonoids, procyanidins, etc.), triterpene saponins, and iridoids exert anti-inflammatory activity without inducing such risks. Due to the extensive content of the subject, the present study focused only on the clinical evidence of several well-known herbal drugs used in arthritic diseases.

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Biomarkers Used in the Diagnosis of Hyper-Inflammation of COVID-19

COVID-19 Hiper-İnflamasyonunun Tanısında Kullanılan Biyobelirteçler

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ABSTRACT Coronavirus-2019 (COVID-19) caused a serious pandemic and killed millions of people. It is expected to have more epidemics in the future due to the ability of rapid mutation of SARS-COV-2 which creates new variants. After the infection starts usually immune response eliminates the virus. However, in some people there is an exaggerated immune-response which leads to hyper-inflammation. This causes more damage in tissues, especially in blood vessels. In extreme cases, disseminated intravascular coagulation occurs and becomes the most important cause of death. Therefore, early diagnosis of hyper-inflammation is very important for timely hospitalization of patients and hyper-inflammation prevention and treatment. This can be achieved by closely monitoring hyper-inflammation biomarkers. It has been shown that lymphopenia, high levels of Interleukin 6, TNF, Interferon I, D-dimer, lactate dehydrogenase, creatinine, cardiac troponin I and myoglobin, prolonged prothrombin and activated partial thromboplastin time are important indicators of hyper-inflammation in COVID-19.

Keywords: COVID-19; inflammation; biomarkers

ÖZET Koronavirus-2019 (COVID-19) dünya çapında büyük bir salgına ve milyonlarca insanın ölümüne yol açtı. SARS-COV-2'nin hızlı mutasyona uğrama yeteneği nedeniyle ortaya çıkan yeni varyantlar ile gelecekte daha fazla salgın yaşanması bekleniyor. Enfeksiyon başladıktan sonra genellikle bağışıklık yanıtı virüsü ortadan kaldırır. Ancak bazı insanlarda aşırı yangıya yol açan abartılı bir bağışıklık yanıtı ortaya çıkar. Bu durum dokularda, özellikle de kan damarlarında daha fazla zedelenmeye yol açar. Aşırı durumlarda yaygın damar içi pıhtılaşma meydana gelir ki bu ölümün en önemli nedeni olur. Bu nedenle hiper enflamasyonun erken tanısı, hastaların zamanında hastaneye yatırılması ve hiper enflamasyonun önlenmesi ve tedavisi açısından çok önemlidir. Bu, hiper-enflamasyon biyobelirteçlerinin yakından izlenmesiyle başarılabilir. Lenfopeni, yüksek İnterlökin 6, TNF, İnterferon I, D-dimer, laktat dehidrojenaz, kreatinin, kardiyak troponin I ve miyoglobin düzeyleri, uzamış protrombin ve aktive kısmi tromboplastin zamanının COVID-19'da hiper-enflamasyonun önemli göstergeleri olduğu saptanmıştır.

Anahtar Kelimeler: COVID-19; enflamasyon; biyobelirteçler

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of coronavirus-2019 (COVID-19) caused a great pandemic with a very high morbidity and mortality. The virus has the ability to mutate very quickly creating new epidemics around the world. Although several effective vaccines have been developed so far it looks like it will be hard to control epidemics due to rapid emergence of new variants which affect people who are previously vaccinated or had COVID-19 infection.[1]

There are not very many effective antiviral drugs that can be used for the treatment of COVID-19. Therefore, it is very important to understand the pathology of the disease and threat the patients by trying to prevent the mechanisms that lead to death. SARS-CoV-2create damage in cells and tissues by two main mechanisms. The first one is by directly killing the cells in which the virus multiplies. The second one is

stimulation of an exaggerated immune response which leads to hyper-inflammation that damages the cells and tissues. This hyper-inflammation is usually the cause of death. Therefore, early diagnosis, prevention and treatment of hyper-inflammation is lifesaving, in critically ill patients of COVID-19.[2-8]

In several studies it has been shown that there are specific biomarkers which are indicators of hyper-inflammation, that should be monitored to diagnose early, this exaggerated state of immune response and try to inhibit the mechanisms that lead to this harmful situation.

Lymphopenia: It has been shown that lymphopenia is an important indicator of the severity of the disease. Lymphopenia usually starts in the second week after the onset of the disease and is strongly associated with high morbidity and mortality. The lower the lymphocyte levels the worse was the prognosis. When the number of different types of lymphocytes were analyzed, it was observed that both helper and cytotoxic T-lymphocytes together with natural killer cells were markedly reduced. However, plasma cells were increased. And among these changes, inflammatory parameters correlated with the reduction of cytotoxic T-lymphocytes.[9-11]

INTERLEUKIN-6 (IL-6)

IL-6 is an important cytokine that regulates acute phase response of the immune system. It is produced by both immune system cells including lymphocytes, macrophages, dendritic cells, mast cells and other cells of connective tissue including keratinocytes, endothelial cells and fibroblasts. IL-6 is a key cytokine causing hyperinflammation in COVID-19. The high level of IL-6 in blood was shown to be strongly associated with the severity of COVID-19. In patients with high IL-6 extensive pulmonary inflammation that led to severe lung tissue damage was observed. Therefore, it is a good biomarker for following the disease progression of COVID-19.[12-16] TNF and IL-1β induce the production of IL-6. IL-6 inhibitors have been shown to decrease the severity and mortality of COVID-19.[17]

TNF

TNF is a proinflammatory cytokine and its production is induced by many disease settings involving acute inflammation. As IL-6, it has been shown that TNF which in turn induces IL-6 production, is also a key player in COVID-19 hyperinflammation. Determination of TNF levels in blood helps predicting the severity of COVID-19. High level of TNF is strongly associated by the mortality.[10,18]

INTERFERON

Interferon-I (IFN-α/β) is a key player in the immune system for initiation of response to many viral infections. It has been shown that Interferon-I production is stimulated by free viral RNA in the blood of COVID-19 patients.[19] Recognition of SARS-CoV-2 RNA by Toll-like receptors (TLR) activates the expression of Interferon-I, which is secreted from infected cells. Interferon-I binds to interferon receptors of neighboring cells and activates several genes via JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling pathway.[20] This creates an antiviral state in the cell. While some studies showed a high level of Interferon-I in severe cases of COVID-19 others showed low levels. The production of Interferon-I in early stages of COVID-19 may stimulate a strong immune response however production at late stages stimulates hyperinflammation worsening the clinical outcome. Therefore, Interferon I levels may not be a good indicator about the severity of COVID-19. The effect of Interferon-II (Interferon-γ) which is considered important for creating antiviral activity increases the production of nitric oxide and induces programmed cell death. Although this response is important at the beginning of the disease, may become detrimental when hyperinflammation starts. On the other hand, high levels of Interferon III, was associated with better outcome. As a summary, Interferon I and III at right level at the beginning of the infection is important for proper antiviral immune response. However, continuation of high levels of Interferon I is one of the factors leading to hyper-inflammation.[21-25]

BIOMARKERS RELATED TO COAGULATION

Endothelial damage and disseminated intravascular coagulation (DIC) are the main causes of bad clinical outcome in COVID-19. Hyperinflammation causes cell damage and starts coagulation. Biomarkers showing the activation of coagulation are important to follow for appropriate clinical management of patients. Fibrinogen level is increased, prothrombin time and activated partial thromboplastin time are prolonged. More important direct indicator of coagulation is elevated D-dimer level which correlates with the severity of the disease and mortality.[26,27]

INDICATORS OF CARDIAC INFLAMMATION AND DAMAGE

Inflammation in myocardium damages myocardial cells and results in the release of their important components into the blood stream. This leads to the increased blood levels of enzymes, lactate dehydrogenase and creatine kinase, together with cardiac troponin I and myoglobin. It is im-

portant to follow these biomarkers during the disease course to diagnose cardiac inflammation early, which may lead to cardiac failure.[28]

FOLLOWING HYPERINFLAMMATION BIOMARKERS FOR THE MANAGEMENT OF COVID-19

In COVID-19 infection damage to tissues are mainly done by two mechanisms. The first one is by multiplication of SARS-COV-2 in the cells. However, the second and maybe more important one is by hyper-inflammation induced by overstimulation of the immune system. In some people, there is a proper antiviral response in the first stage of the disease and the inflammatory response subsides when the multiplication of the virus is eliminated. In some people however, there is an exaggerated immune response triggering more tissue damage and stimulating more inflammation in a vicious cycle. It is therefore important to detect early to transformation to this second hyper-inflammatory stage and try to prevent it by following the hyper-inflammation biomarkers. Many studies indicated that high IL6 levels maybe the first indicator of the start of hyperinflammation. Interferon-I is an important mediator to start an antiviral response. However, high levels of Interferon-I may indicate hyper-inflammation. On the other hand, high level of Interferon-III indicates better prognosis. Although TNF is a less specific indicator for COVID-19 hyper-inflammation, high levels of TNF together with high levels of IL6, indicate disease progression. At this stage, there is usually lymphopenia due to depletion of these immune system cells. One of the worst results of hyper-inflammation is damage to vascular endothelium which stimulates intravascular coagulation. The indicators for this situation are thrombocytopenia, prolonged prothrombin and activated partial thromboplastin time. Specific or general inhibitors of the mediators of hyper-inflammation and prevention of intravascular coagulation, together with supportive treatment against the effects of organ failure constitute the basis of late stage COVID-19 therapy.[10,17-19 29]

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The Therapeutic Effect of Ozone and/or Prolotherapy on the Free Oxygen Radicals

Serbest Oksijen Radikalleri Üzerine Ozon ve Proloterapinin Tedavi Edici Etkisi

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ABSTRACT Investigating the role of ozone therapy and prolotherapy, which are complementary medicine applications as current treatment approaches on free oxygen radicals is extremely significant in the neurosurgical clinic. Ozone (O3) is a gaseous nonradical molecule consisting of three oxygen atoms and is a colorless, pungent odorous molecule that is more unstable than oxygen. The basic logic of ozone therapy, which is one of the regenerative medicine technique, is the development of living tissues that will replace and repair damaged tissues. The other traditional medicine method called as prolotherapy, is to important for improve neurological system and also body health promotion, improve functional capacity of body and reduce pain. Prolotherapy is a medical injection treatment and the injected solution consists of natural sugar or salt water. The body perceives this solution as harmful and sends cells to the injection site that initiate the natural healing process. Prolotherapy injection mimics the natural tissue healing process via strengthening ligaments and tendon structures by stimulating the three phases of tissue healing, namely inflammation, proliferation and tissue regeneration.

Keywords: Free oxygen radicals; ozone therapy; prolotherapy

ÖZET Güncel tedavi yaklaşımları olarak tamamlayıcı tıp uygulamaları olan ozon tedavisi ve proloterapinin serbest oksijen radikalleri üzerindeki rolünün beyin ve sinir cerrahisi alanında araştırılması klinikte son derece önemlidir. Ozon (O3), üç oksijen atomundan oluşan, gaz halinde, radikal olmayan bir moleküldür ve oksijenden daha kararsız, renksiz, keskin kokulu bir moleküldür. Rejeneratif tıp tekniklerinden biri olan ozon tedavisinin temel mantığı, hasarlı dokuların yerini alacak ve onaracak canlı dokuların geliştirilmesidir. Proloterapi olarak adlandırılan diğer geleneksel tıp yöntemi ise sinir sistemi dahil vücut sağlığının geliştirilmesi, vücudun fonksiyonel kapasitesinin artırılması ve ağrının azaltılması için önemlidir. Proloterapi bir tıbbi enjeksiyon tedavisidir ve enjekte edilen solüsyon doğal şeker veya tuzlu sudan oluşur. Vücut bu enjeksiyon ile zerk edilen ajanı zararlı olarak algılar ve doğal iyileşme sürecini başlatan hücreleri enjeksiyon bölgesine gönderir. Proloterapi enjeksiyonu, doku iyileşmesinin üç aşamasını, yani inflamasyon, proliferasyon ve doku yenilenmesini uyararak bağları ve tendon yapılarını güçlendirerek doğal doku iyileşme sürecini taklit eder.

Anahtar Kelimeler: Serbest oksijen radikalleri; ozon tedavisi; proloterapi

THE THERAPEUTIC EFFECT OF OZONE ON FREE OXYGEN RADICALS

Ozone (O3) is a gaseous nonradical molecule consisting of three oxygen atoms and is more unstable than oxygen. Ozone, a colorless and pungent-smelling gas, was used in the first years after its discovery for disinfection, that is, for cleaning microbes. The reason for its disinfecting effect is its strong oxidizing property. It can oxidize all microorganisms and their toxins, that is, it provides disinfection by disrupting their structure. Ozone is a very reactive gas and is toxic to living creatures. The most sensitive organs to ozone are the lungs and eyes.

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The basic logic of ozone therapy, which is one of the regenerative medicine methods, is the development of living tissues that will replace and repair diseased tissues.

Ozone therapy or, as the Germans define it, "Blood washing" is used in many diseases from impotence to stress, from cancer to many infectious processes. In recent years, ozone therapy is on the agenda at the beginning of anti-aging treatments against the effects of aging in the aging process.

During the First World War, Albert Wolf contributed to the application method, which was first started by Dr. Clarke HJ in the 1900s by using ozone in diabetes, anemia, cancer, and morphine poisoning, by treating gangrenous wounds with ozone.[1-4]

In 1980, Horst Kief scientifically announced that he treated AIDS patients with ozone for the first time. While the medical ozone association established in Germany since 1972 continues its work, the Russians have added ozone to their burn treatments since 1992.[2]

Half a billion dollars is spent annually in the USA for ozone treatment. In many countries in the world such as Germany, England, USA, Malaysia, Brazil, there are private clinics that only perform ozone therapy. Today, 8,000 physicians in Germany apply ozone therapy. In Turkey, treatments gained momentum with the work of the Medical Ozone Association, which was established in 2000, and an "Ozone center" was the first established unit by Prof. Avni Babacan in Gazi University in 2011. It is a member of the European Ozone Association and the World Medical Ozone Federation.[3,4]

Frequently asked questions about ozone therapy are as follows: What is ozone therapy, why is ozone therapy important, can healthy individuals receive ozone therapy, is ozone therapy effective in the treatment of cellulite, is it possible to lose weight with ozone, to whom is ozone therapy not applied, is it possible to become beautiful with ozone, are there any side effects of ozone therapy, what is the effect of ozone therapy on sexual function, why is ozone effective in many diseases, does ozone therapy provide support in the process of stress and intelligence activities, what is the effect of ozone therapy on the immune system etc. In this study, all these questions will be answered on the basis of the literature, as well as the relationship between inflammatory and ozone therapy with advanced studies in recent years.

Ozone therapy is a treatment method that uses the "antioxidant" and "anti-inflammatory" potentials of the human body, which are two powerful potentials of the human body, and strengthens the body against diseases by causing an alarm reaction in the whole body. Research over the last 20 years has shown that ozone therapy contributes in many ways to the human body's own therapeutic potential. As a result of this "anti-oxidant" and "anti-inflammatory" response created by repeated ozone therapy sessions, the body's immune cells produce molecules called cytokines. This provides very successful results in the application of medical ozone, especially to patients with weak and impaired immune systems.[5-8]

Ozone was first discovered in 1785 by chemist Martin von Marum. In those years, this gas was noticed for the first time with its sour smell that appeared around various machines that produced electricity.[6]

It was first used on humans in 1840 by the German chemist Schönbein. Physicist Werner invented the machine to produce ozone for the first time in 1857. The principle of obtaining is through the release of ozone after a while in an electrically charged pipe. In 1860, ozone cleaning of the waters was started for the first time in Monaco. It has been understood that ozone contributes to the removal of bad odors and tastes in water as well as cleaning. Ozone in medicine for the first time was used to heal wounds in World War II.[1] In 1916, Dr. Wolff reported astonishing improvements in the healing of serious wounds with microorganisms by the administration of ozone gas. In 1925, Professor Wehrli used both ozone and ultraviolet therapy together for the first time in Italy and named this practice "blood washing". In 1933, dentist Fisch reported that he had good results with ozone in dental diseases. In 1935 Payr, Professor of surgery at the University of Leipzig, presented a study of the positive effects of the clinical application of ozone. In 1926, Dr. Otto Warburg received the Nobel Prize in 1931 for his work that suggested that cancer starts from a lack of oxygen at the cell level. Extending the same work, he went down in history as the only medicine man to receive the Nobel Prize once again in 1944. In 1938, the French surgeon Aubourg presented a study on wounds in the vagina and uterus, and the beneficial results obtained by blowing ozone into the sinus cavity in sinusitis. After the second world war, the beneficial results of ozone were proven in every field, and ozone therapy became widespread using modern devices. In 1979, For the first time, Dr. George Freibott treated an AIDS patient with ozone. In 1980, Dr. Horst Kief argued that ozone was successful in the treatment of AIDS. Ozone therapy has been used as a routine treatment method in burn cases in Russia since 1992.[5,6]

Ozone rapidly transforms into molecular oxygen and oxygen radicals in biological environments and creates a moderate oxidative stress in the body. In this way, ozone is perceived as an oxidative threat in the body. This results in the stimulation of enzymes working in anti-oxidant defense systems.

Like other gases, ozone can dissolve in water. However, unlike oxygen in biological fluids, it quickly reacts with biomolecules. With this reaction, reactive oxygen derivatives and lipid oxidation products are formed. Reactive oxygen spices are rapidly removed from the environment and are responsible for the rapid effect of ozone. Examples of reactive oxygen spices are superoxide, hydrogen peroxide and hypochlorous acid. In particular, hydrogen peroxide acts on erythrocytes, allowing oxygen to be released to tissues more easily, and triggers stimuli that increase the production of various interferons, interleukins and transforming growth factors in leukocyte and endothelial cells. The half-lives of lipid oxidation products can reach hours and are responsible for the long-term effect of ozone. It is possible to see these positive effects of ozone if it is used in appropriate doses. While at high concentrations it is toxic to cells, at low concentrations the expected free oxygen radicals and hydrogen peroxide response may not be sufficient and the desired therapeutic effect may not be seen. The therapeutic concentration of ozone in the studies was determined as 10-80 μg/mL. At the same time, there is an increase in both oxygenase-1 enzyme activity with ozone application and it has been shown that this enzyme has anti-oxidant, anti-apoptotic and anti-inflammatory effects. Ozone makes rapid changes in its location. It increases immunity (immunomodulator), opens blood vessels (vasomodulator) and regulates cellular balance. On the other hand, it accelerates cell proliferation.[6,8,9]

Additionally, it increases the oxygen transport of the blood. It reduces blood coagulation. It activates the production of all biological protectors and enzymes that are vital.

Medical ozone application consists of a minimum of 95% oxygen and a maximum of 5% ozone. Since the 1980s, the use of ozone for medical purposes has increased. Ozone therapy is defined as the application of a certain amount of medical ozone to the body cavities or circulatory system.

Ozone is a special and dense form of oxygen. Chemically, there are 2 atoms in oxygen while there are 3 atoms in ozone. While ozone gas decomposes in 30 minutes at normal room temperature, it splits into monoatomic and diatomic oxygen in 2-3 seconds when given to the blood. Red blood cells absorb diatomic oxygen immediately. Ozone gas has a very high attraction power on red blood cells. Most of the time, the cumulative moving red blood cells, which are attached to each other similar to magnetic coins, immediately separate when they encounter ozone gas, change shape and begin to move faster in regular rows. In addition, as their surface expands, red blood cells that receive a large amount of oxygen can reach the part of the body that they need to be faster. For example; the red blood cells, whose oxygen rate and speed increase with ozone gas, reach the damaged tissues wherever there is a problem and accelerate the body's self-repair capacity. Cells activated by ozone are not only red blood cells, but also white blood cells, which are the body's defense cells. For this reason, it should be accepted that ozone is a natural treatment method that activates the body's own dynamics, unlike other treatments that may have extra and side effects given externally. With its strong oxidizing and effective disinfecting properties, it is used as a germ killer in treatment plants that provide drinking water around the world.[6,10]

It destroys bacteria, viruses, fungi, molds as well as ammonia, sulfur and nitrogen compounds in a short time. Ozone is a disinfectant 3125 times faster and 50% more effective than chlorine. When ozone is given to the body or blood, it immediately dissolves in body fluids 10 times more than oxygen and reacts with ions and biomolecules with its strong oxidant effect.

Ozone shows its effect with hydrogen peroxide formed as a result of these reactions and lipid peroxides, which are lipid oxidation products. While hydrogen peroxide enters blood cells and reveals early biochemical effects, lipid peroxides act on other body cells and reveal long-lasting biochemical effects. For these reasons, ozone is one of the most powerful oxidant molecules known. When applied in low doses, it activates the body's own resistance and activates the immune system. Small immune cells that are activated provide protection against diseases by stimulating the entire immune system through special stimulants. In addition, ozone gas suppresses inflammation by activating the body's own anti-oxidant system. For this reason, it is a complementary medicine application for treatment of chronic diseases. Ozone; is used in the treatment of diabetes, hypercholesterolemia, hypertension and autoimmune diseases including chronic inflammatory processes, relieves muscle and joint pain due to its analgesic properties. It accelerates the healing of tissues by increasing the oxygen capacity of the body. Ozone therapy improves the oxygen needed for the continuation of life to reach the cells more effectively and the capacity to use oxy-

gen more effectively by the cells. It has been determined that the level of lactic acid, which occurs as a result of metabolic activities, is less in people who receive ozone therapy. With ozone treatment, the body uses oxygenated respiration more effectively, making the body more resistant and resistant to diseases. Ozone therapy also shows the effect of increasing physical performance and accelerating metabolism.[6-10]

Today's diet with additives, away from regular sports, and modern life in metropolises surrounded by air pollution cause some harmful effects, especially in all individuals over the age of 40. Lifestyle, foods, light-dark cycle have shown significant changes on our lives. Conditions such as recurrent headaches and body aches, reluctance, weakness, boredom, morning fatigue, and inability to concentrate are complaints arising from the modern world. For these and many other reasons, even healthy people can benefit from ozone therapy. Ozone therapy solves the problems due to atherosclerosis, hypertension, deterioration in blood sugar and blood lipids, etc., which occur due to cellular anti-aging effect in parallel with aging. Ozone therapy is one of the most powerful anti-aging treatments known. It repairs the damage caused by free oxygen radicals on a cell basis and increases the effectiveness anti-oxidants. The increase in the oxygen carrying capacity of the blood means that all organs, including the skin, can benefit more effectively from the nutrients they need. In addition, it also creates a detox effect in the body by removing the toxins accumulated in the body. In this sense, it fights the signs of aging. The fewer toxic substances in the body, the less free radicals are formed. This is the most effective way to stop aging in a cellular sense.

In order to reveal the role of ozone therapy in the aging process, three important factors that initiate aging should be mentioned. These factors are free oxygen radicals, age-related decrease in hormones and enzymes and can be listed as the effect of unhealthy life and wear. Telomere (DNA chain) shortening and telomerase enzyme play a major role in the aging process. Telomeres are caps at the end of DNA that protect the genetic information in DNA. The cell has 23 pairs of chromosomes, each with a telomere at its end. Telomeres shorten with each cell division. Short telomeres are a biomarker for poor health and premature death. The length of the DNA chain at the end of the chromosomes is called telomere, the telomere shortens a little with each cell proliferation, over time the cell cannot reproduce (aging process). It prevents telomere shortening, telomerase enzyme to some extent. The enzyme that uses the RNA it contains as a blueprint, ensuring the protection of telomere ends that shorten with each division. It adds telomeric DNA sequences to the ends of linear chromosomes. The cells that continue to proliferate because there is no telomere shortening: sperm/ovum, stem cells and cancer cells. There are reports that it is possible to stay young and healthy for many years by just lengthening telomeres. For this, it is argued that it is enough to make a few changes in your lifestyle and adopt some new healthy habits. In 2009, Elisabeth Blackburn together with Carol W. Greider and Jack W. Szostak, discovered the role of telomeres and the telomerase enzyme in aging, and immediately after winning the Nobel Prize in Medicine, Blackburn and her team conducted numerous clinical research on lifestyle changes like regular sleep, activity, healthy eating, stress-free life etc., and the effects of these changes on telomeres. It is reported that the change in telomeres lengthened up to 43%.[6-10] The higher the oxidative stress, that is, the free radical rate, the faster the disease and aging. Oxidative stress has been proven to be reduced in people receiving ozone therapy. This effect occurs by the elimination of the effect of free radicals as a result of the increase in anti-oxidant substances in our body. People who receive ozone treatment feel well-being, vigor and vitality. In addition, while people are resistant to diseases, aging is delayed. Free radicals are molecules that usually contain an oxygen atom that has lost an electron. This makes them unstable. This structure causes them to covet the electrons of neighboring molecules. We have a defense system against free radicals formed in our body. This is called the anti-oxidant system. The strongest anti-oxidants of our body are superoxide dismutase, glutathione peroxidase, catalase.[6,7]

Ozone gas has anti-viral and anti-bacterial effects. It is widely used in cleaning wounds and in the treatment of bacterial and viral diseases. It is also applied in circulatory disorders due to its circulatory stimulating effect. Impaired blood circulation of the skin and subcutaneous tissue is known as the most important cause of cellulite. The blood circulation of the adipose tissue is less than the other tissues, and the veins here are under pressure due to the elasticity of the skin. Ozone therapy regulates circulation and supports the vascular system. With ozone therapy, the subcutaneous adipose tissue and the circulation of the skin, which are effective factors in the treatment of cellulite, are significantly improved.

If the body cannot reset the free radicals with these antioxidants in its own body, that is, if the free radical - anti-oxidant balance is disturbed in favor of free radicals, this situation is called "oxidative stress". Oxygen is an in-

dispensable element for the continuation of life. When oxygen is used for energy production, it causes the formation of both reactive oxygen species and reactive nitrogen species, and free radicals are constantly produced by the mitochondria during the body's normal use of oxygen.[1,3,5-7]

These free radicals, which are formed as a result of energy production, can cause changes in the structure of lipids, proteins and nucleic acids.[11] Free radicals are produced from mitochondria as well as from many endogenous and exogenous sources. These produced free radicals have benefits as well as harms. Beneficial effects can be mentioned when free radicals are in low concentration. Low-density free radicals play a role in the activation of cellular signals such as calcium release from intracellular stores, activation of tyrosine amino acid phosphorylation and activation of growth factor signals, along with defense functions such as defense against infections, killing cancer cells and detoxification of xenobiotics. Free radicals are defined as high-energy atoms or molecules that carry one or more unpaired electrons in their outer orbital. Free radicals can easily react with other substances because they have unpaired electrons. Free radicals in the organism can be generated by both endogenous and exogenous sources. Free radicals are produced continuously in the cell and in the environment.[11,12]

Free radicals can disrupt the cell's energy system and cell membrane, disrupt the cell's DNA or RNA structure, and initiate chain reactions that may lead to cell death. It can cause the cell to multiply uncontrollably, causing it to become cancerous and can disrupt normal cellular activities by attacking enzymes and proteins. Such damage to the membranes of the cells lining our blood vessels can lead to atherosclerosis and ultimately to myocardial infarction and strokes. As a result of the attack against the cross-links in the collagen, it can cause hardening in the tissues, decreased performance in the heart, and rheumatic diseases. In studies; it has been shown that free radicals play a role in the formation of osteoporosis, early menopause, coronary heart disease, Diabetes Mellitus, Alzheimer's and Parkinson's disease. The antioxidant defense system is decreased in these patients.[13-15]

Ozone therapy is a reliable treatment method that does not have any side effects if it is performed by a specialist and in expert hands. Ozone therapy is applied via different techniques such as major autohemotherapy and blood applications. Methods of administration include intravenous, intramuscular, intrapleural, rectal, intrarectal, intradiscal, intra-articular, intrapulmonary, and local, topical applications. The classic application of ozone therapy is the application with a needle.

There are five main and most common application methods as major hemotherapy, minor hemotherapy, bagging, rectal administration and intra-articular application.

Major hemotherapy: It is the return of 50-100 mL of blood of the person after mixing with ozone gas. In this application, some blood is taken out of the body, contacted with an oxygen/ozone mixture for 5-10 minutes in an ozone-resistant bottle and then given to the same person again.

Minor hemotherapy: It is the injection of 5-10 mL of blood of the person into the muscle after mixing it with ozone gas. 5 mL of blood and the same amount of oxygen/ozone mixture at a concentration of 80-100 µl/mL are contacted for one minute and injected through the buttock muscle. It is stated that this application regulates and strengthens immunity.

There are situations that should be considered in ozone application. One of these is the prevention of contact with air due to the reactive nature of ozone. In case of contact with air, nitrogen dioxide, a toxic gas, can be formed. Another is that gaseous ozone should not be administered intravenously due to the risk of embolism in the patient during and after ozone application. In addition, the devices used during the application must be resistant to ozone.

Adjuvant or systemic ozone therapy is the return of 50-100 mL of blood of the person after mixing with ozone gas as a major autohemotherapy. In this application, some blood is taken out of the body, contacted with an oxygen/ozone mixture for 5-10 minutes in an ozone-resistant bottle and then given to the same person again.

Bagging is a closed system in which ozone gas is supplied directly to the area to be made locally. It is applied for all kinds of wounds. The area of the wound is taken into an ozone-resistant bag and ozone gas is continuously injected into the bag. Since ozone gas is not absorbed from dry skin, the area to be made must first be moistened with ozonated water.

Rectal administration is the rectal administration of ozone gas. It is preferred in people who have intestinal disease or who cannot have vascular access. The person does not feel discomfort during the application.

Intra-articular application is the direct injection of ozone gas into the joint.

Monthly follow-ups are recommended after 12-14 sessions of ozone therapy, the session of which lasts for

30-45 minutes on average. Therapy sessions start with twice a week and as it progresses, the session intervals are opened and reduced to once a month.

Side effects have been listed as follows: In the absence of an appropriate dose, treatment cannot be beneficial. After 3-5 sessions of ozone treatment, complaints such as fatigue, headache and weakness, which are called 'healing crisis', can be seen in the person. These complaints disappear when the treatment is continued.

Due to the increase in ozone applications in recent years, the number of cases reporting complications has increased in the literature. However, complications of ozone application are limited to local injection application. Apart from this, it should be avoided in some cases. These situations are; glucose-6-phosphate dehydrogenase enzyme deficiency, pregnancy, those receiving angiotensin converting enzyme inhibitor therapy, hyperthyroidism, bleeding disorder, uncontrolled cardiovascular disease and asthma patients that react to ozone. The most important of these is the case of fatal septic shock after paravertebral muscle application. Complication is possible due to violation of the rules of asepsis.

Contrendications are as follows: Uncontrolled hyperthyroidism, those who have advanced anemia and blood related diseases such as hemophilia, bleeding coagulation disorder, those with Favism disease, which is accompanied by glucose-6-phosphate dehydrogenase enzyme deficiency in white blood cells, in patients with brain diseases in which bleeding continues actively and in cardiac infarctions in the acute stage. No treatment should be done in the first three months of pregnancy unless it is necessary. Although ozone therapy has no known harmful effects, it is not recommended during pregnancy, especially since it is a critical developmental period for the baby.[16-23]

Today, ozone is used in many fields of medicine in general. Application areas can be listed as follows: Neurological/orthopedic diseases (sports injuries, fibromyalgia, general condition improvement, arthrosis/discopathy etc., infectious diseases (viral, bacterial parasitic diseases), autoimmune diseases (rheumatoid arthritis, Crohn's disease, celiac disease etc.), cardiovascular and circulatory diseases (coronary artery, peripheral artery, cerebral artery atherosclerosis), degenerative diseases (senile dementia, macular degeneration, arthroses), lung diseases (COPD, asthma bronchiale), dermatological diseases (psoriasis, dermatitis, acne etc.), oncology (as chemotherapy and radiotherapy support), gastrointestinal and urogenital diseases (ulcerative colitis, proctitis, prostatitis etc.), wound treatment. Recently, it is generally used in many fields of medicine like toothache and infections, wounds that are difficult to heal, such as diabetic foot, subacute and chronic low back pain, pain treatment and anti-inflammatory effect in degenerative joint diseases etc. The number of both experimental and clinical studies investigating the results of anti-inflamatuar effect of ozone therapy to date is even limited.[8,9] Recently, resarchers announced that, future analysis of ozone effects needs to incorporate more global evaluations of inflammation, airway hyper-responsiveness, and epithelial damage.[9]

Further clinical end experimental studies involving both human and animal participants are now needed in order increase our knowledge of evidence-based ozone therapy protocols for treating many inflammatory and also ischemic status.

THE THERAPEUTIC EFFECT OF PROLOTHERAPY ON FREE OXYGEN RADICALS

Prolotherapy as named in Webster's Third New International Dictionary is "the rehabilitation of an incompetent structure, such as a ligament or tendon, by the induced proliferation of new cells."[24,25] The name prolotherapy comes from the word "proli-" (Latin) meaning offspring; "proliferate"to produce new cells in rapid succession. Prolotherapy is a low-risk injection therapy that uses the body's self-healing mechanisms to improve structure and function via strengthening ligaments and tendon structures.[25-29] Because prolotherapy is a treatment modality that may provide a solution to a patient's pain symptoms, it may be beneficial prior to long-term opioid therapy or surgical intervention.[25] Prolotherapy, is an injection-based complementary and adjunt medical therapy for chronic musculoskeletal problems. The aim of prolotherapy is the resolution of both pain and functional loss, and also the optimizing of body health by the individual regaining the ability of daily activities and ability of exercise.[30,31]

There are many different prolotherapy application practice for patients. These range from hypertonic dextrose or sugar (12.5-25%) solution, subcutaneous via subcutaneous injections prolotherapy, platelet-rich plasma (concentrated platelet cells from the patient's own blood) prolotherapy, prolotherapy using stem cells and bone marrow or adipose (adipose) tissue for all other cell types of the human body.[32,33]

Prolotherapy involves the injection of solutions at which tendons and ligaments inserting points to the bone to cause an inflammatory reaction. Inflammation initiates the

self regeneration and repair of the injured tissue in and around the joint to promote tissue repair or growth.[25] Three solutions are commonly used in prolotherapy as D-glucose (dextrose), phenol-glucose-glycerin, and sodium morrhuate. These three solutions are generally used individually and are hypothesized to create local irritation and subsequent inflammation and anabolic response.[34]

Basicly, the concept of prolotherapy is the regeneration and repair of tissue by inducing inflammation using irritants. Recent study has shown that 10% dextrose appeared to result in the repair of articular cartilage defects in experimental animal study.[35-37] In the other study, reserchers have also demonstrated that 20% dextrose exerted a regenerative effect in the healing of injured Achilles tendons in rats through the proliferation of fibroblasts. In human *in vitro* studies, a high glucose environment resulted in increased production of platelet-derived growth factors by the vascular endothelium and mesangial cells.[37-39]

Dextrose prolotherapy and saline prolotherapy involve injecting a solution containing irritants as a saline or dextrose solutions into a specific area (knee, wrist, ankle etc) where damage or injury has comprised.[30-33,35,36] Prolotherapy shows its effect through reducing pain and stiffness, improving strength, function, and mobility of the joint, increasing the strength of ligaments and other tissues. Prolotherapy was induced inflammation of injured ligaments will lead to self-repair.

The theory of prolotherapy was searched and applied as early as the fifth century B.C. by Hippocrates. Hippocrates would treat unstable joints by cauterizing the ligaments using a hot metal rod.[30,31] Hippocrates hypothesized that causing inflammation of injured ligaments would lead to self-repair.[25] This was one of the initial steps towards using the body's own healing mechanism to repair connective tissues. For the first time, Pmeroy conducted research on the history of prolotherapy and has written a book. Hackett and Shuman has begun injections into the ligaments in the 1930s. It has been used for nearly 100 years, however, its recent applications can be traced to the 1950s when the prolotherapy injection protocols were formalized by George Hackett who is a general surgeon in the U.S, based on his clinical experience of over 30 years. Hackett published his first prolotherapy book in 1956. In his book, prolotherapy is done via fibroblasts and by stimulating osteoblasts adhesion of tendons and ligaments to bone a place that provides strength (weld) defined as a technique injured ligament, tendon to joints various irritant inject substances.[29,40-42]

Prolotherapy has traditionally treated weak human ligaments with various sclerosing or as a technique of strengthening proliferating solutions with injections known and often hypertonic dextrose, sodium morrhuate, phenol, a saline solutions is used. Injections typically with connective tissue dysfunction applied to or near the injured area.[29,35,36,40-42] This injection technique was first developed by Louis Schultz in 1937 has been revealed. Schultz, painful temporomandibular joint subluxation into this joint space 0.25-0.5 mL sodium psyliate (psyllium extract) were effectively treated with injection. Moreover, Schultz has shown that after injection of sodium psyliate, the soft tissue fibrosis develops within 4-6 days via the experimental study. These findings indicate the "sclerosing effect" of the injected solutions and also strengthens the intra-articular ligaments and joint capsule. It has been shown that it can be beneficial in providing joint stabilization.[35,36,43] This idea was generalized by George S. Hackett. Chronic painful musculoskeletal disability is often caused by ligament laxity and that this situation causes joint instability and subsequent cause activation of nociceptive fibers in ligaments and tendons. Physical trauma as potential sources of ligament laxity stated that abnormal posture and age-related degenerative changes may occur.[26-29,41,42] Nociception due to mechanical instability of secondary tissue ischemia and thus causing a sensory vicious circle is considered also abnormal caused by prolonged muscle spasms. It is suggested that these forces will increase tendon or ligament laxity. In this situation, mechanical instability further increases and a vicious circle is formed.[44]

Prolotherapy is likely to be safe, as long as the medical doctor has training and experience in these types of injections and also has prolotherapy sertificate. However, there are risks involved with injecting substances into a joint. Possible adverse effects are pain and stiffness, bleeding, bruising and swelling infection, allergic reactions. Depending on the type of prolotherapy, less common adverse effects are spinal headache, spinal cord or disk injury, nerve, ligament, or tendon damage, a collapsed lung, known as pneumothorax. During the procedure, the doctor cleans the skin with alcohol applies lidocaine cream to the injection site to reduce pain injects the solution in the affected joint.

The process takes around 30 minutes, including preparation, after the patient arrives at the facility.

Immediately after treatment, the doctor may apply ice or heat packs to the treated areas for 10-15 minutes. During this time, the patient rests. Then the patient be able to go home. Immediately after the application, the patient

likely notices some swelling and stiffness. Most patients can resume daily activities by the day after, although bruising, discomfort, swelling, and stiffness may carry on up to a week. According to an article published in the Journal of Prolotherapy, for an inflammatory condition involving a joint, the typical method of treatment is three to six injections at intervals of 4-6 weeks. For neural prolotherapy, such as nerve pain in the face, the typical method of terapy is weekly injections for 5-10 weeks.[3,4,16,20,25]

Although inflammation is normally a pathological condition, the inflammatory reaction is physiologically the body's cellular damage and also the first response injury. Triggering inflammation is both microorganism (gram-positive and gram-negative bacteria, virus, fungus, parasite, etc.) and non organismal causes (trauma, burn, foreign body, ischemia, pancreatitis, etc.), the cellular response of the body is the same.[8,9,12,13]

The aim of prolotherapy are to improve support of health, improve functional capasity, and reduce pain. After a careful history and physical examination, those cases who have undergone conventional medical management may benefit from this type of treatment. Conventional treatments for back pain are rest, ice/heat, professional or home physical therapy or/and exercise program, behavioral modifications, and non-opioid pain medications.[45]

In 1989, Klein et al. reported conservative treatment and patients with chronic low back pain whose complaints persist despite laminectomy. A study investigating the effectiveness of prolotherapy injections on they have conducted. In this study, 6 weeks of prolotherapy was given to all patients. Spinal mobilization was applied with the injection and lumbar flexion/extension exercises are given. In the post-treatment evaluation. Significant improvement in VAS pain and disability scores compared to pre-treatment detected.[46]

In 2003 Yelland et al. investigated the effects of exercise on chronic nonspecific low back pain. In the study they conducted, prolotherapy (20% glucose, 0.2% lignocaine) was administered to 110 patients via administered saline injection on two study groups. Both of these groups are flexion, by randomized to extension exercises and normal activity groups has been created.[47] Significant improvements were found in disability scores.[47] The overall conclusion showed that, when combined with spinal manipulation, and exercise, prolotherapy may improve chronic low back pain and disability. The current most promising indication for prolotherapy appears to be in the treatment of tendinopathies. Early studies indicate promising results in the treatment of osteoarthritis, but further clinical trials are needed before recommendations can be practice.[48,49]

Blood contains endogenous growth factors and therefore prolotherapy may have potential implications for applications. After epidural injections autologous blood patches are often used to prevent spinal fluid from flowing. In a study they conducted to investigate the effects of conservative treatment extensor carpi radialis brevis in unresponsive patients with chronic lateral epicondylitis. 2 mL of autologous blood, 1 mL of 2% lidocaine and 1 mL of 0.5% bupivacaine under the tendon. Significant reduction in mean pain scores with the injection of the resulting mixture have reported.[35,36,50]

In 2006, Dagenais et al. researched and published the most common side effects of prolotherapy injection treatment. They found that the pain as the side effect with the highest mean prevalence (70%) detected. Besides, the stiffness (25%), skin injury (5%) and bruising (5%) was observed. Further, possible side effects might be a headache, nausea, diarrhea, and minor allergic reaction to the injected agent.[45,51-53] The patients can return to daily activities after injections.[53,54] Rare injuries include lung collapse, disc injury, meningitis, hemorrhage, and nerve damage.[51,52]

The contraindications to prolotherapy are nonmusculoskeletal pain, metastatic cancer, septic joints or cellulitis, systemic inflammation, spinal anatomical defects, morbid obesity, bleeding disorders, chemical dependence, low pain threshold, and whole body pain.[45,51,52]

Most recently, prolotherapy has been used to treat many professional athletes such as football players.[45] Recent findings and long-lasting experience in this field supported prolotherapy as a technique to manage osteoarthritis, musculoskeletal pain, joint pain, and laxity, chronic low back pain, lateral/medial epicondyle overuse tendinopathy, and other related musculoskeletal problems safely.[53]

The use of regenerative injection therapies will carry on to expand with further research, advances in treatment strategies, and an increasing demand for techniques such as prolotherapy, platelet-rich plasminogen (PRP), and stem cell treatments for different types of pain treatment.[45]

As a result, when the up-to-date methods and treatment modalities on the subject are examined in the literature, it is necessary to briefly mention the concept of prolazone, which is on the agenda by providing new and combined treatment content. Prolozone is a technique that recently practice as combination concepts from neural therapy, prolotherapy, and ozone therapy. It involves injecting various combinations of procaine, anti-inflammatory med-

ications/homeopathics, vitamins, minerals, proliferatives, and a mixture of ozone/oxygen gas into degenerated or injured joints, and into areas of pain. The result of the procedure is nothing short of remarkable in that damaged tissues can be regenerated, and otherwise untreatable pain can be permanently healed.[55,56]

As a conclussion, according to my clinical experience, both ozone therapy and prolotherapy, separately or together, provide a good application option for the treatment of intradiscal disc protrusion under scopy without open surgery. Thus, geriatric patients with comorbidities have the chance to be treated without hospitalization and also without the risks and complications of anesthesia and open surgery.

Further case control clinical end experimental studies involving both human and animal participants are now needed in order increase our knowledge of evidence-based ozone therapy and prolotherapy protocols for treating many traumatic, inflamatory and also ischemic problems. It focuses on human needs, as the aim of medicine is to keep the individual in a state of complete emotional and physical well-being. In this respect, ozone therapy and prolotherapy, which are traditional medicine methods, are among the treatment options to meet this need with their effective role in the free oxygen radicals. Scientific data on these treatment modalities and relation with free oxygen radicals should be supported by further researches.

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Complementary Medicine Modalities: The Effect of Acupuncture Treatment and Mesotherapy on Free Oxygen Radicals

Tamamlayıcı Tıp Modaliteleri: Akupunktur Tedavisi ve Mezoterapinin Serbest Oksijen Radikalleri Üzerine Etkisi

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ABSTRACT The word acupuncture is a combination of the words "acus" (needle) and "puncture". The effects of all types of acupuncture are pain-reducing, relaxing, providing homeostasis, raising the immune system, and also psychological, repairing and regenerative effects. The basics of acupuncture are that, acupuncture plays an important role for attenuation of the levels of inflammatory cytokines such as IL-1b after surgery and in reducing the incidence of fever of unknown origin. The effect against free oxygen radicals has been proven with acupuncture treatment. The use of acupuncture as an adjunct treatment to conventional medical therapy for many chronic inflammatory disease and autoimmune problems seems acceptable in all over the world. Mesotherapy is a word derived from the Latin words "meso" and "therapy". Mesotherapy, which literally means "medium treatment", is a treatment method that has medical and aesthetic treatment applied to the mesoderm layer of the skin. Classical pharmacological therapy to reduce pain, inflammation, and functional disability usually relies upon the extensive use of nonsteroidal anti-inflammatory agents, paracetamol, corticosteroids, and various different kind of opioids. In contrast to mesotherapy, major drawback of pharmacological therapy with these types of analgesics and anti-inflammatory drugs is the frequent related with adverse effects for the treatment of acute pain and sensory symptoms of musculoskeletal disorders. As a conclussion, the use of acupuncture and mesotherapy as an adjunct treatment to conventional medical therapy for many chronic inflammatory disease and autoimmune problems seems acceptable and should be validated by confirming its evidence-based applicability via many further comparative case control studies.

Keywords: Acupuncture; mesotherapy; free oxygen radicals; inflammation

ÖZET Akupunktur kelimesi "acus" (iğne) ve "ponksiyon" kelimelerinin birleşiminden oluşur.Tüm akupunktur yöntemlerinin etkileri ağrıyı azaltıcı, rahatlatıcı, homeostaz sağlayıcı, bağışıklık sistemini yükseltici, ayrıca psikolojik, onarıcı ve yenileyici etkilerdir. Akupunkturun temelleri, akupunkturun cerrahi sonrası IL-1b gibi inflamatuar sitokinlerin düzeylerinin düşürülmesinde ve nedeni bilinmeyen ateş insidansının azaltılmasında önemli bir rol oynamasıdır. Akupunkturun birçok kronik inflamatuar hastalık ve otoimmün problem için geleneksel tıbbi tedaviye ek bir tedavi olarak kullanılması tüm dünyada kabul edilebilir görünmektedir. Akupunktur tedavisinin serbest oksijen radikallerine karşı etkisi kanıtlanmıştır. Mezoterapi, Latince "mezo" ve "terapi" kelimelerinden türetilmiş bir kelimedir. Kelime anlamı olarak "orta tedavi" anlamına gelen mezoterapi, derinin mezoderm tabakasına uygulanan medikal ve estetik tedavilerin olduğu bir tedavi yöntemidir. Ağrıyı, inflamasyonu ve fonksiyonel sakatlığı azaltmak için klasik farmakolojik tedavi genellikle nonsteroidal anti-inflamatuar ajanların, parasetamolün, kortikosteroidlerin ve çeşitli farklı opioidlerin yaygın kullanımına dayanır. Mezoterapiden farklı olarak, bu tür analjezikler ve anti-inflamatuar ilaçlar ile farmakolojik tedavinin en büyük dezavantajı, kas-iskelet sistemi bozukluklarının akut ağrı ve duyusal semptomlarının tedavisi için sık görülen yan etki profilleridir. Sonuç olarak, akupunktur ve mezoterapinin birçok kronik inflamatuar hastalık ve otoimmün problem için geleneksel tıbbi tedaviye ek bir tedavi olarak kullanılması kabul edilebilir görünmektedir ve kanıta dayalı uygulanabilirliği daha çok karşılaştırmalı vaka kontrol çalışmasıyla doğrulanmalıdır.

Anahtar Kelimeler: Akupunktur; mezoterapi; serbest oksijen radikalleri; inflamasyon

THE EFFECT OF ACUPUNCTURE TREATMENT ON FREE OXYGEN RADICALS

Nowadays, the adjunt complementary therapies such as acupuncture, ozone therapy, prolotherapy, mesotherapy and even nutritional support have a very wide application area. One of the goals of adjunt complementary medicine is to improve the quality of life. Acupuncture and moxibustion (treatment with heat) are explained at length in the book "HUANG DI NEI JING", which was written 4700 years ago during the Yellow Kingdom of China.[1,2]

In addition, this book gives detailed information about channels and points, needles, manipulation techniques and diseases that acupuncture treats. "Huang Di Nei Jing" is considered as the first medical book. In Europe, the first books on acupuncture were written in the 1600s. Acupuncture is hypothesized to have its origins in blood ritual, magic tattooing, and body piercing related with shamanic healing performed during the Neolithic Age (8000-5000 BC) for the aim of reorganizing health and reliability and protection against demonic infestation and malevolence.[1-3]

In 1972, US President Richard Nixon made an official visit to China with a large delegation. During this visit, Chinese doctors showed the American delegation "a surgical operation performed under acupuncture anesthesia". After this event, the popularity of acupuncture increased in the West; Its application and examination has become widespread all over the world.[2,3]

Acupuncture and traditional oriental medicine is a science proven by statistics approved by the World Health Organization (WHO). There are chairs that teach acupuncture in 90 universities in many european countries and 47 states of the USA, Canada and Mexico. It works with a total of 19 centers in 10 countries around the world such as China, North Korea, South Korea, Japan, Vietnam, Belgium, Romania, Italy, Sudan and the USA as Traditional Eastern Medicine. In recent years, acupuncture is accepted as an adjunt complementary medical method applied by inserting special needles with very fine tips into certain points of the body and is an important component of traditional Chinese medicine. The word acupuncture is a combination of the words "acus" (needle) and "puncture". According to traditional Chinese philosophy, man is considered a part of the living universe. It is believed that the universal power that is present in everything is also in the human being. This power or "energy" called "CHI" circulates in the human body in channels called meridians.There are 14 meridians in the human body (12 double and 2 single). According to the belief in China, "CHI" (energy); YIN and YANG are under the influence of universal forces. In traditional Chinese medicine, health is seen as the result of the energy balance in the body called "chi". When this energy balance is disturbed, health deteriorates and disease occurs. In our body, there are 12 double and two single meridians, which are classically located on 360 acupuncture points. Acupuncture refers to certain points of the body along the meridians.[1,2]

Classic body acupuncture is applied to acupuncture points using the special needles on the energy meridians circulating in the body and dates back to before Christ. It is a form of treatment that is given by warning with very fine-tipped special different length and size needles. In the use of the defined unit of measurement, acupuncture points are defined through obvious anatomical structures (eg bone protrusion, body-midline). According to Chinese medicine, needling an acupuncture point according to a precise localization on the surface, taking into account the necessary puncture direction and immersion depth, provides the emergence of a feeling called "De chi" in accordance with the character of the point.[1,2]

Electro acupuncture which is providing electric stimulation at acupoints on the body is used to give constant standardized additional stimulation. Intradermal manuel acupuncture is used to give continuous stimulation at acupoints to consolidate the curative effect. Electro acupuncture generates CXCL 10 at the site of inflammation and induces antinociception via peripheral opioid peptides.[1,3,4]

Treatment using acupuncture laser is another form of application used in recent years. Advantages of laser acupuncture treatment over conventional needle acupuncture are absence of aches and pains, no risk of infection, babies and children are comfortable during therapy and can be used safely, treatment time is shorter. Acupuncture treatment has disadvantages compared to classical modern medical methods. Laser acupuncture treatment may not reach deep acupuncture points, the other is the large number of acupuncture sessions.[5]

Ear acupuncture is a method that was discovered after the second world war and applied according to the rules of the French Doctor Nogier. As a result of his research, Doctor Nogier concluded that in the 1950s, the body was represented in the auricle as lying in the uterus position.[6]

The effects of all types of acupuncture are pain-reducing, relaxing, providing homeostasis, raising the immune system, and also psychological, repairing and regenerative effects. Acupuncture is preferably single-use,

sterile and stainless, with the patient in the supine position in the form of inserting steel or sterile gold and silver needles into selected application areas is applied. The exact localization of the spots at the level of the skin surface is achieved here using the Cun-measure, which is a relative measure and corresponds to the width of the thumb of the person to be measured. Mild numbness, pain, known as De-qi sensation in Chinese medicine in patients while being immersed, discomfort occurs. In fact, this feeling is one of the findings showing that the needle is in the right place. Needles are left for about 20-30 minutes at short intervals throughout treatment. The patient can be warned by needle movement. Needles are removed at the end of the treatment. When we insert the needle into the acupuncture point, the nerve (receptor) is stimulated. This stimulus reaches the spinal cord via the nerve and from there to the necessary centers in the brain. In both body and auricular acupuncture, the points have electrically low skin resistance and high skin conductivity. If a pathology has occurred in the body, the electro-dermal activity of the acupuncture point is more increases.[1-6]

Dr.Paul Nogier has revealed that all organs in the body have reflection areas in the auricle and when these areas are combined, the shape of an upside down fetus in the uterus is revealed. According to Overbye's theory, first the organ develops in the embryo and at the end of the 7th day following this development, the reflection (communication) area in the auricle is formed as a result of a synchronization between the embryological development of the organs and the auricle parts. According to the result of this theory, ear acupuncture can be effective in treatment because there is synchronization in the formation of somatotopy in the ear. The whole body is represented in the auricle, according to Nogier's somatotopic fetus model. The front of the ear is sensory and the back is motor. As a result, various chemical substances are secreted in different areas of our body. Chemical substances secreted through the circulation reach the diseased area and show their effect.[1-4]

Meridian theory is an important part of Chinese medicine and has guided acupuncture and clinical practice for thousands of years. Many important concepts about human body function and regulation rules have been defined. But when compared to the basic concepts of modern medicine, it has been shown to have great differences. These differences both caused deep debates and aroused interest in scientific circles. According to studies supporting the physical reality of acupuncture practice, the following mechanisms come into play: Stimulation of Aβ fibrils in the skin and muscle that transmit impulses to the spinal gray matter, reducing pain sensation by inhibiting painful stimuli from the periphery, release of β-endorphin and met-enkephalin in the brain, activation of descending pain control systems in the midbrain, finding a regulatory effect on the central pain network in the limbic system and hypothalamus, acupoints have low electrical resistance, spread of heat and sensation after needling, radioisotope trace studies, discovery of connective tissue-matrix-meridian and points, confirmation of Bonghan channels with Tc99m imaging, and also histological studies.[1,2,7,8] The National Acupuncture Meridians Research Institute, led by Dr.BH Kim, conducted research on the anatomical structure and physiological state of AP and AM in 1963 using animal staining techniques. β-endorphin increase in cerebrovascular fluid (CSF) has been shown in the treatment of pain with acupuncture, as well. Acupuncture is ineffective in the area of the nerve blocked by local anesthesia.[8]

Acupuncture points are the exit points of nerves, arterioles and venules that penetrate the fascia. Of the 409 points, 386 were found very close to the main blood vessels.[9] Acupuncture points and their relationships with meridians are quite complex. Although it seems that the studies are completely based on interactions in the somatic nervous system, the point of interest should be the interactions between the autonomic and somatic nervous system and the conduction pathways in the autonomic nervous system. The sympathetic nervous system is of great importance in the effects of acupuncture. The first study on this subject Matsumoto & Hayes showed a decrease in blood pressure and intestinal vasodilation following electroacupuncture application in rabbits.[10] Like blood vessels, the lymphatics are accompanied by very fine nerve fibers, such as the onset of pain following an untreated hand infection.[11,12]

According to the Bonghan theory, there is a circulatory system of its own, completely different from the blood and lymphatic circulation and nervous systems. This system consists of threadlike channels (Bonghan ducts, BHD) similar to acupuncture meridians and specialized tissue bundles (Bonghan corpuscles, BHC) corresponding to acupuncture points. Bonghan fluid flows within the BHD meridians. This liquid is produced by BHC and plays an important role in the formation of the therapeutic effect in acupuncture treatment by carrying electrical and mechanical stimuli.[13,14] By analyzing the fluid, Kim and colleagues observed that it contains important components such as hyaluronic acid, neurotransmitter hormones (adrenaline, noradrenaline), amino acids and free nucleotides. An im-

portant function of this fluid is to transmit electrical signals over the BHD network.[15] In recent years, the Bonghan research team has found specific dyes for imaging these structures in blood and lymphatic vessels. This specific dye is Alcian blue and is a very common cationic dye for the demonstration of glycosaminoglycans.[16,17] The discovery of the Bonghan system has provided important information in many ways to explain the medical and biological significance of the practice of acupuncture. For example, the circulatory function of acupuncture therapy, electrophysiological excitability function, hormonal effect, effects on immune function, effects on hematopoiesis function,as well as regeneration and virtual (microcell) effects on obesity and on the relationship between cancer. The mechanism of effect can be defined as the local cellular stimuli starting from the stimulated point reaching the brain via neural communication. This effect is transmitted from the brain to the relevant organs. Thus, chemicals, hormones and enzymes that already exist in our body are secreted. Since some biochemical-cellular changes are caused, our body's immune system becomes active and healing is achieved through "self-healing power". Thanks to these effects of acupuncture, many pharmacologically effective secretion of the substance is stimulated. Substances such as endorphins, serotonin, prostaglandin, and GABA also have an effect on immunity. Each types of organisms which can damage the organs, tissues and cells on human body, like toxins and tumors all of the body's defense system against cells and activities for resistance and destruction is defined as immunity. The effects of acupuncture on immunity are observed in cancer studies. Interleukin 2 (IL2) level and natural killer (NK) in cancer patients cell activity is found to be lower than normal. These rates were significantly higher after treatment. It has been found to increase in healthy individuals in the control groups, there is no change.[18] In cancer patients, T3 (OKT3+), T4 (OKT4+) and T8(OKT8+) lymphocytes are found lower than normal. These rates increased significantly with treatment. The increase in T4 lymphocytes is higher than the others. In healthy individuals in the control groups, there is no change.[18,19] Acupuncture theraphy had been given to 90 patients as a study group, who applied to the pain clinic for the pain is not relieved by medication in France between 1999 and 2000. As a result of the study lasting 2 months, the patients' pain evaluated according to VAS criteria and 36% decrease detected. Patients with cancer-related pain from acupuncture have been found to provide a very significant benefit.[19,20]

Inflammation is a cellular, humoral and vascular serial vital response that living tissue gives to any living or non-living foreign factor or internal/external tissue damage. Although inflammation is normally a pathological condition, the inflammatory reaction is a physiological response of the body. The immune system controls every foreign substance which enters the body or contact. This system removes them from the healthy body cells of the living thing and distinguish it from its healthy tissues. Innate and adaptive immune system in humans; to special functions organs, many different cells and is a complex system of molecules. For this reason, the inflammation that occurs after the organism's exposure to the damaging substance and the tissue repair that develops after it occurs in the living thing. It is one of the most complex processes to come defense mechanism that can be activated after damage occurs. It plays a critical role in the survival of living things. Inflammatory response to microbes and damaged tissue not only destroys harmful substances, but also activates the repair process. Although inflammation is normally a pathological condition, the inflammatory reaction is physiologically the body's tissue damage and also the first response to injury. Triggering inflammation is both infectious (gram-positive and gram-negative bacteria, virus, fungus, parasite, etc.) and non infectious causes (trauma, burn, foreign body, ischemia, pancreatitis, etc.), the answer is the same. Inflammation occurring in any part of the body has characteristically five symptoms. Pain in this area, increase in temperature, redness, edema and loss of function constitute the five characteristics of inflammation. The role of "Capicolaminas" and "Cerotoninas", which are cerebral neurotransmitters effective in acupuncture analgesia, is being investigated. The release of these neurotransmitters can produce analgesic, muscle relaxation, anti-inflammatory, mild anxiolytic and antidepressant effects. Endogenous opioid peptides such as beta endorphine are associated with acupuncture analgesia.[21]

Opioid-containing leukocytes migrate to the inflamed tissue and secrete opioids such as endorphin, met-encephalin and dynorphine.[21] Thus, it creates antinociception by binding opioid receptors on peripheral nociceptive neurons.[21] There is evidence to support the role of endogenous opioids (encephalin, dynorphin, endorphin, and endomorphin) and serotonin (5-HT) in acupuncture analgesia.[22]

Mayer et al. and Pomeranz et al. showed that acupuncture analgesia was blocked with naloxone in mice and humans, supporting the role of endogenous opioids.[23]

Possible mechanisms of acupuncture for attenuating inflammation have been evaluated to date by a number of researchers. The stabilizing, anti-inflammatory and anti-

depressant effects of acupuncture have also been accepted by WHO, and this method, which stimulates the body's healing mechanisms and does not cause side effects, ensures that patients are protected from the side effects of long-term drug use.[24]

The higher the free radical rate, the faster the disease and aging. The effect against free oxygen radicals has been proven with acupuncture treatment. This effect occurs by the elimination of the effect of free radicals as a result of the increase in antioxidant substances in our body.

According to traditional Chinese medicine, the lung and spleen are two organs that take part in the removal of facial deposits and the destruction of toxic substances. By using the points on the lung and spleen meridians, water and blood chi are activated in the applied area. People who receive acupuncture treatment feel well-being, vigor and vitality. In addition, while people are resistant to diseases, aging is delayed. Depression and anxiety are reduced. Sleep pattern returns to normal.The digestive system is regulated. It has a positive effect on hot flashes. It reduces the frequency of headaches and also body pains. Acupuncture is completely safe. It is a painless method. No recovery period is needed. It is economical method. It is a completely natural form of treatment. Indications are listed below: protection from side effects of chemotherapy such as nausea, protection from the side effects of radiotherapy such as dry eyes and mouth (xerostomia, xeropthalmia), fatigue, insomnia, pain control, conditions such as depression and distress, endocrine disorders, cancers and also hot flashes in menopause.

Contraindications are pregnancy (except some acupuncture points), emergencies, bleeding diathesis. Complications are rare. Transient ecchymoses, which sometimes last up to a week locally, hypotensive attack and syncope are possible complications.

Up to date, there have been many acupuncture researches conducted involving cases after surgery but most of them have focused on controlling pain, postoperative nausea and vomiting, and alleviating intra/postoperative immunosuppression.[25-27] There are limited studies available on the effects of acupuncture on the anti-inflammatory response after surgery.[28] In the rat study with our study group, we applied electro-acupuncture therapy to the responses related to the inflammatory process in the ovarian ischemia-reperfusion model, as well.[29] In the past years, the only treatment option applied when a necrotic and swollen ovary was salpingo-oophorectomy. Hovewer, in the last twenty years, even the swollen and purple-blue necrotic ovaries can be preserved without surgery. In such cases, conservative treatment is recommended.[30] After starting use of detorsion treatment for adnexal torsion, the subject of ischemic injury came to questionnaired. Tissue oxidative products accumulate in the interval between torsion and detorsion. The products damage the ovarian tissue following reperfusion.[31] Consequently, investigatorrs have started to explore various agents, plant extracts, or adjent/complamentery technics for reversing such reperfusion ovarian injury without resulting in ovarian follicular depletion.[29] In our study, the first of its kind in the literature, was investigated the protective effect of electroacupuncture in a rat model of ovarian ischemia-reperfusion injury resulting in decreased ovarian reserves. Our results suggest that the acupuncture points studied may be effective in ameliorating the micro-anatomical and biochemical effects of ischemia on the ovary in terms of ovarian reserve markers.[29] We claimed that studying oxidative stress parameters, which are already investigated in such study, is an inefficient use of resources, since the main aim in case of torsion is to prevent apoptosis as a result of accumulating oxidative stress. In addition to apoptosis, is the level to which ovarian reserve markers are directly affected.[29,30]

In literature there are few review articles present the evidence that the anti-inflammatory pathways of acupuncture are mediated via there flexive central inhibition on the congenital immune system.[3,21,29,30] Both *in vitro* and *in vivo* clinical trials have recently represent the existence of a negative feedback loop between the autonomic nervous system and the congenital immunity.[3] The cholinergic anti-inflammatory pathway affords basic, conjunctive, and integrative medical evidence for the systemic immunoregulatory actions of acupuncture and auriculotherapy, in view of which, their use as a supplementary therapy to conventional medical treatment for a number of cytokine-mediated diseases is plausible. The use of acupuncture as an adjunct treatment to conventional medical therapy for many chronic inflammatory disease and autoimmune problems seems acceptable and should be validated by confirming its evidence-based applicability. Ulett and Han have further suggested that certain homeostatic effects of acupuncture and auriculoterapy might be directly explained by the "broad parasympathetic effects" of the vagus nerve.[3,32]

The basics of acupuncture are that, acupuncture plays an important role for attenuation of the levels of inflammatory cytokines such as IL-1b after surgery and in reducing the incidence of fever of unknown origin.[28] The mechanism of acupuncture and other nonpharmaceutial therapies are related with an increase in the actions of the

efferent vagus nerve system.[3] Efferent activity in the vagus nerve causes acetylcholine release in organs of the monocyte-macrophage system, where ACh binds to the α7 nicotinic receptors on tissue macrophages and inhibits the release of proinflammatory cytokines.[3]

As a conclussion, the use of acupuncture as an adjunct treatment to conventional medical therapy for many chronic inflammatory disease and autoimmune problems seems acceptable and should be validated by confirming its evidence-based applicability via many further comparative case control studies. World's leading hospitals complementary medicine in their experts on patients evidence-based and complementary medicine by physicians offers applications. As a result of the stimulation and regulation of the autonomic nervous system, reparative substances are activated and the mechanisms to solve the skin problems begin to work. In summary, acupuncture treatment increases the number of leukocytes. It helps the patient cope with stress. It strengthens the organs and regulates the body balance. It reduces toxic accumulation. It helps in mental balance. It reduces nausea and vomiting. Provides analgesic effect. It corrects sleep disorders.

THE EFFECT OF MESOTHERAPY ON FREE OXYGEN RADICALS

Mesotherapy is a word derived from the Latin words "meso" and "therapy". Mesotherapy, which literally means "medium treatment", is a treatment method that has medical and aesthetic treatment applied to the mesoderm layer of the skin. Dermis, below the surface ectoderm of the skin, originates from the mesenchyme of mesoderm (or center germ cell layer).[33] Mesoderm forms muscles, sex organs, outer surface of internal organs, inner skin, bone and cartilage, heart and blood vessels.[33]

It is a procedure that uses a needle to the middle layer of the skin, a method similar to acupuncture. With the help of needles, amino acids, minerals, enzymes and vitamins can be given to the middle layer of the skin, sometimes alone or sometimes mixed with each other, as well as opaque non-steroidal anti-inflammatory, myorelaxant, etc. injectable agents with appropriate dilution rates can be injected into this layer for medical treatment or eastethic purposes. Since its presentation during the 1950s created by French doctor Michel Pistor, the utilization of mesotherapy has produced a lot of enthusiasm among clinicians and patients. Dr. Michel Pistor, detailed empowering results with little dosages of drugs controlled intradermically to cases with an assortment of clinical conditions. The Italian Society of Mesotherapy (SIM) was determined in 1975 with the essential goal of assessing and approving mesotherapy in Italy utilizing logical methods.[34,35] Mesotherapy treatment is a non surgical restorative preparation pointed toward reducing issue, for example, cellulite, abundance weight, body weight, and face and neck revival, cosmetically. It is directed by means of various infusions containing different kinds of FDA endorsed prescriptions, nutrients, and minerals, medically.[34] Numerous fine infusions of natural concentrates, drugs (non-steroidal anti-inflammatories, myorelaxants, etc.) and an extraordinary "cocktail" arrangement of nutrients (e.g. pseudocholinesterase, caffeine, L-carnitine, etc.) are infused into the problem spot and re-mixed at regular intervals. The fat is broken down and consumed through the lymphatics and transported to the liver, where it is removed from the body via feces.[34,35]

The indications for mesotherapy are trigeminal neuralgias, cervicobrachial neuralgias, pain, stiffness that will occur after joint degeneration, swelling and limitation of movement, De Quervain's disease, Dupuytren's contracture, tendinitis, teosynovitis, bursitis hygromas, redness and movement of connective tissue pathology, limitation, hydrolipodystrophies-diffuse cellulitis, pain in acute and chronic soft tissue strains, redness and limitation of movement, myofascial pain syndrome, migraine headaches, in edema due to micro-circulatory disorder, as a supporter for healthy people, skin pathologies such as keloid, alopecia, acne supportive in the treatment, pain due to spasmodic pathologies, strengthening the immune system, soft tissue sports injuries such as arthritis, osteoarthritis, rheumatoid polyarthritis, acute rheumatism, degenerative diseases, joint pathologies, arteritis, micro-circulation problems, gynecology and obstetrics, vascular pathologies, such as hypertension, hemiplegia, cerebral palsy, assisting general rehabilitation practices in diseases can be used as a treatment modality. The treatment regimen to be applied for each disease condition is different. Selected treatment regimens for several conditions with inflammatory process are briefly exemplified below. For performing mesotherapy in tenosynovitis; NSAID, an anti-edematous and proteolytic enzyme mixture (diluted with SF) is applied to the damaged area with 15 intradermal microinjections, 0.1 mL to each point. The application is repeated in sessions on the 7th and 14th days. The patient who suffer from lumbal osteoarthritis, a combination of procaine, pentoxifylline, Vitamin B1 can be applied via PPP application with 6/13 mm mesotherapy needle. Then procaine, pentoxifylline and baclofen combination can be applied via Napaj. In the next sessions, it can be treated with procaine, pentoxifylline and calcitonin as also Napaj application.

Mesotherapy is applied in the form of manual and device application. Manual mesotherapy application method can be listed as intra-epidermal injection, superficial intradermal injection, deep intradermal injection, hypodermal injection, traditional mesoperfusion and combined techniques. In the manual epidermeal technique, 1 mm is inserted into the epidermis using a 13 mm 30 G needle. In the deep intradermal injection technique, 1-3 mm. without forming deep papules (0.1-0.2 mL) drug is given to each point. It is made by the PPP method. Hypodermal injection can be applied to a depth of 4-13 mm using PPP method. In the traditional mesoperfusion method, each 6 mm. 6 needles in length to give one drop of each drug every 2 seconds. It is in the form of application in more than 10 minutes. Combined techniques are the Napaj (superficial epidermal application)together with the injection a point-to-point (PPP) intradermal application.

Device application tecniques are Burst technique (napage with injection device), controlled one shot (point to point/PPP), continuous injection (point to point/PPP), long term injection (mesoperfusion).

The issues to be considered in the selection of the drug to be used in the mixture can be listed as follows: All products must be sterile and water-soluble products, non allergic and isotonic, subcutaneous tissue tolerance should be excellent (neutral pH), tissue integrity must be complete, the mixture should be clear, the pH of the cocktail products is close to each other, compatible, precipitate should not create, there should be no antagonistic effects between products, there should be a synergistic effect, reconstitute with local anesthetic or SF. The issues that the practicing physician should pay attention to can be listed as follows: The substances to be injected should be supplied in advance and should not contain cosmetic topical content. The cosmetic products should be Class III injectable form. Intramuscular administration should be diluted and isotonic. Thus, necrosis is also prevented. Substances should be soluble in water and not soluble in oil. Physical and chemical incompatibilities should be eliminated. Mixture substance number should be reduced preferably not more than 3. In medical mesotherapy application, for example, between diclofenac sodium and procaine and with ketoprofen. There is an incompatibility between the solvent and procaine. Therefore, SF should be used as a solvent instead of the drug's own solvent. Mixtures should be clear.

Compared to other medical methods, the advantages of the mesotherapy method can be listed as follows: The drug is released into the connective tissue. Access of the product to the tissue not subject to personal changes. In perderma, the rate of release of drugs remains the same for everyone. The drug reaches the diseased area without any change. Medications that cannot be taken due to their side effects may be given. Other advantages can be listed as being economical, non-toxic, applicable near the lesion, safe to use in cases where cortisone is contraindicated, no general intolerance, reaching the site of action in the shortest time and providing a long-lasting effect due to its slow intradermal absorption.

For ideal results, at least 4-6 sessions are required. Significant results can be seen after one month of treatment. Because it depends on the patient's body, the amount of medication required differs from patient to patient.[34,36] Despite the fact that mesotherapy just includes micro-injections, these should in any case be managed as effortlessly as painless as could reasonably be expected during application. The determining factors in this regard, apart from the dynamic agents, are the infusion procedure and the nature of the syringes and needles. All materials must be disposable silicone and latex. Accessible needle designs incorporate the 1 mL, 2.5 mL, 5 mL, 10 mL and 13 mL sizes.[34,37]

It is thought that the intradermal more superficial injection of the solutions used into the target tissue allows it to be destroyed by the blood circulation from the target tissue later and therefore to remain in the tissue for a longer period of time. The dermis layer of the skin is rich in capillary blood vessels and lymphatic vessels and nerve fibers. A reservoir environment is created for molecules injected into the dermis layer of the skin as papules. These injected molecules are distributed to the tissue. Microcirculation is stimulated. Low-density molecules pass into the systemic circulation by capillaries, and high-density molecules by lymphatic vessels. As the injections are made superficially, the residence time of the given substances for instance non steroid anti-inflamatory in the tissue can be prolonged at that rate. The higher the oxidative stress, that is, the free radical rate, the faster the disease and aging. Oxidative stress can proven to be reduced in people receiving mesotherapy.[37-40]

Mesotherapy has a very wide application purpose and application area. Various factors such as the person's health status, age, physical characteristics, need status are the conditions that change the content to be injected with the microinjection application There are many findings in the literature regarding the anti-inflammatory effects of mesotherapy. A growing body of literature supports the efficacy of mesotherapy, particularly in the therapy of musculoskeletal pain. Administration of conventional NSAIDs by mesotherapy is effective in managing bursitis, tendinitis etc in osteoarthritis in the short term and mid-

term. Non-operative management is usually the first line of treatment for inflamatory based disorders and includes generally oral non-steroidal anti-inflammatory drugs, injections of medications, rehabilitation techniques and also mesotherapy. These observations can be of interest for efforts to reduce the adverse effects of oral administration of anti-inflammatory drugs. The results of the studies confirm the efficacy and tolerability of anti-inflammatory/analgesic administered as local intradermal therapy which is known as mesotherapy. The cause of cellulitis is also caused changes in the microcirculation and chronic oedema (as a result of structural changes in the adipose cells and the formation of micronodules) are important predisposing factors. Active substances that affect the microcirculation administered intradermally produce clinical improvements in patients with vascular problems and chronic oedema.[35,38]

Side effects in mesotherapy can be examined in three groups as drug dependent side effects, depend on the mode of administration, and connected to the input of the needle. Contraindications of mesotherapy are episode after syncope, open wounds, end-stage heart failure, Diabetes Mellitus, patients under anticoagulant treatment, kidney failure, in patients with hypersensitivity to drugs and pregnancy.[34-38]

Medical classical pharmacological therapy to reduce pain, inflammation, and functional disability usually relies upon the extensive use of nonsteroidal anti-inflammatory agents, paracetamol, corticosteroids, and various different kind of opioids. However, the major drawback of pharmacological therapy with these types of analgesics and anti-inflammatory drugs is the frequent related with adverse effects. Low-dose anti-inflammatory drug injection via mesotherapic methods and also myorelaxant via mesotherapy may be a complementary option alongside traditional approaches for the treatment of acute pain and sensory symptoms of musculoskeletal disorders such as low back pain, chronic venous lymphatic insufficiency, oedematous fibrosclerotic panniculopathy (more commonly known as cellulite), intradermal vaccination, and in the management of skin aging.[35,41]

Further case control clinical end experimental studies involving both human and animal participants are now needed in order increase our knowledge of evidence-based mesotherapy protocols and to compare mesotherapy to conventional approaches for treating many inflamatory and also ischemic problems. Scientific data on mesotherapy modalities and relation with free oxygen radicals should be supported by further researches.

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