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Assessment of Bone Marrow Biopsy and Cytogenetic Findings in Patients with Multiple Myeloma
Multipl Miyelomlu Hastalarda Kemik İliği Biyopsisi ve Sitogenetik Bulguların Değerlendirilmesi

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Abstract
Objective: Multiple myeloma (MM) is a malignant condition that is characterized by the accumulation of malignant plasma cells. Although MM remains incurable, the survival of MM patients has improved considerably due to applied autologous stem cell transplantation (ASCT), novel agents, and treatment strategies. This study aimed to determine the cytogenetic characterization and bone marrow features of Turkish patients with MM.
Materials and Methods: Eighty-five MM patients were admitted to the 9 Eylul University Hospital in Turkey. Bone marrow (BM) samples MM patients were subject to cytogenetic analyses on diagnosis and during therapy as part of therapeutical and clinical evaluation. A complete cytogenetic study was performed using the G-banding technique. The Fluorescent in situ hybridization (FISH) analysis was performed using cytoplasmic immunoglobulin (cIg)-FISH. The degree of bone marrow fibrosis was determined using the histochemical stain of

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reticulin. We determined the percentage of bone marrow plasma cells based on the extent of CD38 staining.
Results: Eighty-five MM patients were retrospectively identified between 2015 and 2021. The median age was 63 (38-90) years. Of the 85 patients, 60 (70.6%) were male, and 25 (29.4%) were female. Seventy-two (84.7%) cases had bone marrow fibrosis at the time of diagnosis. The most common was grade-2 fibrosis, recorded in 35 patients (41.2%). About 72.9% of the patients showed more than 50% plasma cells. The FISH analysis indicated the presence of abnormal chromosomes in 37% (32/85) of the patients. The most frequent abnormality was IGH translocation (21.3%).
Conclusion: Subgroup analysis of IGH mutations is crucial in the identification of high-risk MM patients. We believe that our study will contribute to the determination of bone marrow biopsy and cytogenetic features of MM patients in our country.
Keywords: Multiple myeloma, Bone marrow biopsy, Cytogenetic analysis, Fluorescent in situ hybridization

Öz
Amaç: Multipl miyelom (MM), malign plazma hücrelerinin birikmesi ile karakterize malign bir durumdur. MM tam kür sağlamanan hastalık olmasa da, uygulanan otolog kök hücre nakli (ASCT) ve yeni ilaçlar ve yeni tedavi stratejileri nedeniyle MM hastalarının sağkalımı önemli ölçüde gelişmiştir. Bu çalışmada multipl miyelomlu Türk hastalarda sitogenetik karakterizasyon ve kemik iliği özelliklerinin belirlenmesi amaçlanmıştır.
Gereç ve yöntem: Türkiye'deki 9 Eylül Üniversite Hastanesi'ndeki 85 multipl miyelom hastasını kaydettik. G-bantlama tekniği kullanılarak tam bir sitogenetik çalışma gerçekleştirildi. Fazlar arası FISH analizi, sitoplazmik immünoglobulin (cIg) -FISH ile yapıldı. Kemik iliği fibrozunun derecesi, retikülinin histokimyasal boyası kullanılarak belirlendi. Kemik iliği plazma hücrelerinin yüzdesi, CD38 boyamasınına göre belirlendi.
Sonuç: 2015 ve 2021 yılları arasında geriye dönük olarak 85 multipl miyelom hastası belirlendi. Ortanca yaş 63 (38-90) yıldı. 85 hastanın 60'ı (% 70.6) erkek, 25'i (% 29.4) kadındı. Tanı anında kemik iliği fibrozu 72 olguda (% 84.7) mevcuttu. En sık 35 hastada (% 41,2) derece 2 fibrozis görüldü. Hastaların% 72.9'unda plazma hücre yüzdeleri% 50'den fazlaydı. FISH analizi sonuçlarında kromozom anomalilerinin varlığı %37 (32/85) oranındadır. En sık kromozom görülen anormallik IGH translokasyonuydu (% 21.3).
Sonuç: IGH mutasyonlarının alt grup analizi, yüksek riskli MM hastalarının belirlenmesinde kritik öneme sahiptir. Çalışmamızın ülkemizdeki MM hastalarının kemik iliği biyopsisi ve sitogenetik özelliklerinin belirlenmesine katkı sağlayacağına inanıyoruz**.**
Anahtar Kelimeler: Multiple miyelom, Kemik iliği biyopsisi, Sitogenetik analiz, Floresan in situ hibridizasyon

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Introduction

Multiple myeloma (MM) is a malignant condition that is characterized by the accumulation of malignant plasma cells. MM is the second most common hematological malignancy that develops in the bone marrow (1). Although MM remains incurable, the survival of MM patients has improved considerably due to the application of autologous stem cell transplantation (ASCT), novel agents, and treatment strategies. However, this development has not been uniform. This heterogeneity depends on patient-specific factors such as age, comorbidity, and disease-related factors, including cytogenetic and molecular features, bone marrow fibrosis (BMF), and the plasma cells in the bone marrow. Several risk classifications have been made for personalized therapeutic approaches. First, the clinical staging system for MM was developed by Durie and Salmon in 1975 (2). Then, the International Staging System (ISS) score was defined by Greipp et al. in 2005. ISS depends on two parameters, serum β2-microglobulin level and serum albumin (3). Avet-Loiseau et al. and Cavo-Rosinol et al. combined cytogenetics with ISS to improve risk stratification (4, 5). Several techniques are available to detect genetic abnormalities in MM. Karyotyping attempts to detect cytogenetic abnormalities and abnormalities dependent on the proliferative index of malignant plasma cells but provides limited information in vitro due to the low proliferative ability of malignant plasma cells (6). Kishimoto, reported that abnormal karyotypes accounts for approximately 30%–50% of the MM cases (7). The gold standard test for the detection of genomic abnormalities in MM is FISH, which has been implemented and validated by global cytogenetic laboratories (8). Identification of high-risk subtypes of myeloma is vital. According to the data, high-risk MM patients showed a 20-50% reduction in overall survival (OS) compared to standard-risk patient groups using induction with conventional

chemotherapy followed by ASCT. This study aimed to determine the cytogenetic characters and bone marrow features of Turkish patients with MM.

Materials and Methods

From January 2015 to January 2021, 85 MM patients were enrolled at the 9 Eylül University Hospital of Turkey. Bone marrow (BM) samples MM patients were subject to cytogenetic analyses on diagnosis and during therapy as part of therapeutical and clinical evaluation. Conventional cytogenetics were made on all samples at presentation and after a 24h/72h short-term culture period. Cells were treated with colcemid and harvested for 20 min. Then karyotype was analyzed using the standard G-banding technique. A minimum of 20 metaphases per case was analyzed. The karyotypes were presented reported according to the International System for Human Cytogenetic Nomenclature ISCN 2016. The interphase FISH study was performed on bone marrow samples according to the manufacturers' hybridization and post hybridization stringency conditions with minimal modifications. Dual-color Cytocell FISH probes specific to loci 13q14.3 (LPH006), CKS1B (1q21 22)/ CDKN2C (1p32.3) (LPH039), IGH (14q32.33) (Break-apart, LPH 014), P53 (17p13.1)/ATM (11q32) (LPH 052), MLL (11q23.3) (Break-apart, LPH 013) were used. Threshold values of copy number gain, deletion, and break-apart positivity are 7%, 7%, and 10%, respectively. FISH slides were analyzed with a motorized Olympus BX61 fluorescence microscope equipped with DAPI, FITC, rhodamine, dual, and triple band-pass filters set (Chroma, USA). Two independent observers analyzed the slides blindly, enumerating at least 200 optimally hybridized nuclei. The degree of bone marrow fibrosis was detected using the histochemical stain reticulin. The level of fibrosis determined by the pathologist was scored according to the guidelines of the World Health Organization (grade 0: no fibrosis, grade 1 (mild): low (fine reticulin network), grade 2 (moderate): intermediate (multifocal or diffuse non-confluent fibrosis), grade 3 (severe): high (marked and diffuse fibrosis). The percentage of bone marrow plasma cells was determined based on the extent of CD38 staining as group 1 (<20%), group 2 (20-50%), and group 3 (> 50% plasma cells). The International Staging System (ISS) was used based on the combination of serum β2 microglobulin and serum albumin.

Statistical Analysis

The statistical software SPSS for Windows version 24.0 (IBM SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. Numerical variables were presented as median/mean, while the categorical variables as number (n), and percentage (%). The overall survival (OS) curves were estimated using the Kaplan–Meier method. OS was defined as the time from the diagnosis of MM to death from any cause. The confidence interval (CI) was determined as 95% in the analyses. P-value of <0.05 was considered to be statistically significant.

Results

Eighty-five multiple myeloma patients were retrospectively identified between 2015 and 2021. The median age was 63 (38-90) years, and 56.5% of the patients were under 65 years old. Of the 85 patients, 60 (70.6%) were male, and 25 (29.4%) were female. Amongst the enrolled patients, bone lesions were detected in 57.6%, anemia in 42.4%, impaired renal function in 27.1%, and hypercalcemia in 18.8% of the cases. Most patients were in stage III of the ISS. More than half of the patients received proteasome inhibitor-based therapy for induction. Information about the induction therapy of five patients could not be obtained. Further details about the demographic, laboratory, and clinical characteristics are presented in Table 1. Bone marrow fibrosis at the time of diagnosis was observed in 72 cases (84.7%). The most common finding was grade-2 fibrosis in 35 patients (41.2%). In 72.9% of the patients, a plasma cell percentage a plasma cell percentage of over 50% was observed, and the most common type of plasma cell infiltration was the diffuse involvement pattern (50.6%). Based on the FISH analysis, 37% (32/85) of the patients showed the presence of chromosomal anomalies. The most frequent abnormality was IGH translocation (21.3%). Based on the

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karyotype analysis, 54.4% (37/68) of the patients had a normal karyotype, while 5.8% (4/68) had an abnormal karyotype. Table 2 represents the plasma cell percentage, plasma cell infiltration pattern, and fibrosis in the bone marrow biopsy samples. Detailed cytogenetic results of the MM patients are presented in Table 3. In this study, the median OS of the entire population was 29.4 months (Figure 1a). Del (17p13) negative patients had a higher OS compared to del (17p13) positive patients. (32.4 months vs. 11.1 months ;P=0.015) (Figure 1b).

IGH negative and del-13q negative patients had a higher OS compared to IGH positive and del-13q positive patients. However, this was not statistically significant (p=0.594 and p= 0.094, respectively). A lower percentage of plasma cells in the bone marrow was associated with a better OS than a high percentage. The median OS was 42.6 months in patients with 0-19% plasma cells, 33.5 months in patients with 20-49% plasma cells, and 28.4 months in patients with 50-100% plasma cells. However this observation was not statistically significant (p=0.969). The results of multivariate analysis adjusting for the del (17p13), IGH, and del-13 mutation, bone marrow fibrosis, the plasma cell infiltration pattern, and plasma cell percentage are shown in Table 4.

Discussion

Multiple myeloma (MM) remains an incurable malignancy with varying clinical and prognostic differences depending on several factors, including age, comorbidity, cytogenetic and genomic features, and tumor microenvironment characteristics in the bone marrow. The disease incidence ranges from 5 to 7 per 100,000 and increases with age. The median age at diagnosis of MM is 69 years. Less than 14% of patients are younger than 55 years. MM is more seen in men and African Americans. The etiological reasons behind these differences are not known enough. MM's survival ranges are from 1 year to more than ten years. Median survival in unselected patients with MM is three years. The 5-year relative survival rate is 46.6% (9). In our study, the median age was 63, and the male patients constituted 70.6%. These findings were slightly different from those published in the literature, maybe due to environmental and genetic factors. Understanding the alterations in the bone marrow microenvironment and the molecular pathways related to these changes are crucial for the further improvement of myeloma treatment and the outcomes of patients with MM. Sailer et al. demonstrated that the the characteristics of the tumor microenvironment such as plasma cell differentiation, the volume of plasma cell infiltration, and the infiltration pattern, have predictive values (10). However, the prognostic efficacy of bone marrow fibrosis (BMF) is still controversial at the time of diagnosis (11, 12). Babarovic et al. reported that the 5-year survival rates of patients with increased BMF subjected to post-treatment bone marrow biopsy were significantly shorter than those without fibrosis. Thus, the assessment of BMF had a prognostic value in the follow-up of MM patients (13). A recent study by Paul and colleagues showed that the median progression-free survival (PFS) in patients without MF was 30.2 months and in those with BMF was 21 months. On the other hand, the median OS was 61.2 months and 45.1 months in patients without and in patients with BMF respectively. These findings were statistically significant (14). Our results suggested that the pattern of plasma cell infiltration and the degree of fibrosis at diagnosis did not significantly influence the survival times, possibly due to the relatively small groups. Several predictive models like Durie-Salmon, ISS have been used to identify myeloma patients in risk groups. The Durie-Salmon staging system is the first for MM and has been widely used for several decades (2). Later proposed by Greipp et al., the ISS used the Durie- Salmon stratification system more widely. The ISS is based on the prognostic factors of serum β2 microglobulin and serum albumin. According to ISS stages I, II, and III, the median of survival MM patients were 62 months, 44 months, and 29 months, respectively (3). A major limitation of the ISS classification is that it does not comprise patient-specific factors. Approximately half of our

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patients were in ISS stage III , and their OS was 28.4 months. Our results were consistent with the study of Greipp et al. Chromosomal abnormalities detected by FISH were combined with ISS to improve risk stratification (4, 5). Because the karyotype is highly sensitive in detecting numerical chromosomal abnormalities, the patients can be classified into specific policy categories as normal, hyperdiploid, or non-hyperdiploid (including hypodiploid, pseudodiploid, and near-tetraploid) karyotypes (15). 15 Among the non-hyperdiploid MM, the hypodiploid subtype is associated with a poor prognosis (16,17). In our study, abnormal karyotypes were detected in 4 of 68 (5.8 %) patients. Among these, non-hyperdiploid abnormalities were observed in two patients. However, chromosomal abnormalities in 39.7% of the patients could not be detected because of the absence of metaphase cells. FISH does not require metaphase cells before analysis, and it's accepted as the gold standard test for the detection of genomic abnormalities in MM, as it is applied and verified by global cytogenetics laboratories (8). Studies are underway for optimal risk-based classification and treatment in MM, especially for the identification of high-risk MM patients. The International Myeloma Working Group (IMWG) has provided a consensus wherein FISH panel testing should be performed for at least t (4; 14), t (14; 16), and del (17p13) to define patients with high-risk diseases (18). Apart from these recommendations, Boyd et al. suggested that the FISH panel test + 1q and t (14; 20) should be performed in MM risk classification (19). Deletion of chromosome 17p13 increases the immortalization of tumor cells by inhibiting apoptosis (20). The incidence of del-17p13 is observed in about 10% of the MM patients and has been associated with impaired OS (21-24). In our study, the incidence rate of the del (17p13) mutation was 7.1% and, del (17p13) negative patients had a significantly higher OS compared to the del (17p13) positive ones (32.4 months vs. 11.1 months respectively). Previously, del13q was in the poor prognosis group but is now excluded from risk stratification, as it was detected in almost 50% of the myeloma patients and is thought to be secondary to the close association with t(4;14) del-17p (19, 25, 26). In this study, the chromosome del13q was detected in 9 out of 49 cases (18.4%), and a significant adverse effect of del13q on OS was not observed (p= 0.094). Chromosome 14q32 translocations (heavy chain immunoglobulin (IgH) translocations) in MM increase dysregulated proliferation and inhibit differentiation. Bergsagel et al. have reported an incidence of IgH translocations of 60-65% in intramedullary MM (27). However, in our study, the incidence of IgH mutations was lower (21.3%). Over 20 different IgH translocations have been revealed, among which IgH translocations, t(4;14), t(14;16), and t(14;20) are associated with poor prognosis in MM patients (28). The use of proteasome inhibitor-based induction is recommended to improve the prognosis of patients with t (4; 14) but not those with del (17p13) (29). Unfortunately, our study does not include subgroup analysis of IGH mutations which involve several recurrent translocations**.** Recently; gene expression profiling, such as, sequence comparative genomic hybridization (CGH), single nucleotide polymorphism sequence CGH, gene expression profile (GEP), and RNA sequencing has been introduced to identify patients with particularly high-risk MM (30-32). However, this is not feasible in routine practice due to the high cost. The present study has a few limitations. It was a retrospective study based on single-center data. Possibly, because of the small sample size, we could not observe a significant adverse effect of IgH mutation on OS. Since our study did not include subgroups of IgH mutations, high-risk patients could not be determined clearly.

To the best of our knowledge, this study is the first in our country to include genetic results. It is especially crucial in the identification of high-risk myeloma subtypes. The subgroup analysis of IGH mutations is critical identifying high-risk MM patients. IGH subgroup analyzes have been conducted recently in our center. Our study will contribute to determining the bone marrow biopsy and cytogenetic features of MM patients in our country.

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Table 1. Demographic, Laboratory, and Clinical Characteristics of Myeloma patients	
Characteristics	n (%)
Age Median (years- range)	63 years (38-90)
<65 years	48 (56.5)
≥65 years	37 (43.5)
Sex	
Female	25 (29.4)
Male	60 (70.6)
Immunoglobulin isotype	
Ig G	48 (56.5)
Ig A	13 (15.3)
Light chain MM	24 (28.2)
Type of light-chain	
Kappa	54 (63.5)
Lambda	31 (26.5)
ISS stage	
I	21 (24.7)

II	23 (27.1)
III	41 (48.2)
Anemia	
Hemoglobin >10 g/dL	49 (57.6)
Hemoglobin <10 g/dL	36 (42.4)
Renal impairment	
Creatinine ≤2 mg/dL	62 (72.9)
Creatinine >2 mg/dL	23 (27.1)
Hypercalcemia	
Serum calcium <11 mg/dL	69 (81.2)
Serum calcium >11 mg/dL	16 (18.8)
Bone Lesion	
Absent	36 (42.4)
Present	49 (57.6)
High ESR Level (>15 mm/h)	
Absent	26 (30.5)
Present	52 (61.1)
Missing	7 (8.4)
High LDH Level (>250 U/L)	
Absent	60 (70.6)
Present	25 (29.4)
High Ferritin Level (>335 ng/mL)	
Absent	51 (60.0)
Present	17 (20.0)
Missing	17 (20.0)
Treatment type used for induction	
VAD	4 (4.70)
Proteasome inhibitor	54 (63.5)
Immunomodulatory drug	3 (3.52)
Both proteasome inhibitor and immunomodulatory drug	19 (22.3)
Missing	5 (5.88)
Total	85 (100)

International staging system (ISS), Erythrocyte sedimentation rate (ESR), Lactate dehydrogenase (LDH), Vincristine doxorubicine dexamethasone (VAD).

Table 2. Analysis of plasma cell percentage, plasma cell infiltration pattern, and fibrosis in bone marrow biopsy samples from patients with myeloma at diagnosis

Bone marrow plasma cell percentage (%)	n (%)
0-19	5 (5.9)
20-49	62 (72.9)
50-100	
Pattern of plasma cell infiltration	33 (38.8)
Interstitial	9 (10.6)
Nodular	
Diffuse	43 (50.6)

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Bone marrow fibrosis	
MF- 0	13 (15.3)
MF-1	35 (41.2)
MF-2	9 (10.6)
MF-3	
Total	85 (100)

Marrow fibrosis (MF)

Table 3. Detailed cytogenetic results of multiple myeloma patients.

Characteristics	Information available (n)	Positive patients n (%)
Del (17p13) Mutation	85	6 (7.1)
Del(13q14) Mutation	49	9 (18.4)
IGH translocations	80	17 (21.3)
CKS1B Mutation	1	0 (0.00)
MLL Mutation	15	0 (0.00)
Karyotyping	68	
Normal		37 (54.4)
Hyperdiploidy		2 (2.9)
Non-Hyperdiploidy		2 (2.9)
Nondiagnostic		27 (39.7)

Table 4. Multivariable Cox regression analysis for overall survival

Characteristics	Median OS (mo)	Hazard Ratio	95% CI	p-value
Del-17p13				0.015
Del-17p13 Negative	32.4	1		
Del-17p13 Positive	11.1	3.45	1.18-10.0	
IGH translocations				0.594
IGH Negative	28.9	1		
IGH Positive	22.8	1.35	0.44-4.11	
Del-13q mutation				0.094
Del-13q Negative	28.4	1		
Del-13q Positive	23.3	1.88	0.89-3.96	
Bone marrow fibrosis				0.938

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MF- 0	34.4	1		
MF- 1	33.0	0.90	0.35-2.29	
MF- 2	27.4	1.15	0.46-2.87	
MF- 3	33.4	1.08	0.31-3.72	
Pattern of plasma cell infiltration				0.466
Interstitial	33.4	1		
Nodular	23.3	1.80	0.69-473	
Diffuse	28.4	1.12	0.55-2.27	
Bone marrow plasma cell percentage (%)				
0-19	42.6	1		0.969
20-49	33.5	0.86	0.23-3.31	
50-100	28.4	0.97	0.29-3.26	

Confidence interval (CI), Marrow fibrosis (MF), Immunoglobulin heavy chain (IGH), Overall Survival (OS).

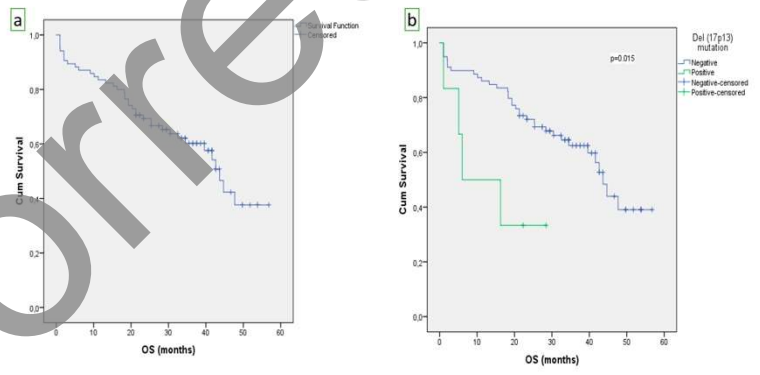


Figure 1. a) Kaplan-Meier curve of OS for the entire study cohort (n = 85). The median OS was 29.4 mo, b) Del 17p13 negative patients had a higher OS compared to Del 17p13 positive patients. (32.4 mo. Vs. 11.1 mo. P=0.015).
Abbreviations: OS=overall survival, mo=months.

uncorrected proof