RESEARCH PAPER

Serum Amyloid A Levels and Severity of COVID-19 in Children

SELIN TASAR,[1] EDA KARADAG-ONCEL,[1] AYSEGUL ELVAN-TUZ,[1] ASLIHAN SAHIN,[1] GULNIHAN USTUNDAG,[1] YILDIZ EKEMEN-KELES,[1] SELIN ONUR,[2] BANU ISBILEN-BASOK,[2] AHU KARA-AKSAY,[1] DILEK YILMAZ-CIFTDOGAN[1,3]

[1]*Department of Pediatric Infectious Diseases, University of Health Sciences, Tepecik Training and Research Hospital, Izmir, Turkey.*
[2]*Health Sciences University, Izmir Tepecik Training and Research Hospital, Medical Biochemistry; Izmir, Turkey.*
[3]*Department of Pediatric Infectious Diseases, Izmir Katip Celebi University, Izmir, Turkey.*

Correspondence to: Dr Selin Tasar, Department of Pediatric Infectious Diseases, University of Health Sciences, Tepecik Training and Research Hospital, Izmir, Turkey. sselintasar@gmail.com
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Objective: The aim of this study was to determine the availability of serum amyloid A (SAA) in the diagnosis of coronavirus disease 2019 (COVID-19), to asses disease severity and to predict hospitalization status. **Methods**: Between March, 2020 and March, 2021, a total of 80 children (40 cases with COVID-19 and 40 cases in healthy group) were included in this study. Patients were divided into two groups (mild and moderate/severe) to evaluate SAA levels in terms of clinical severity and also hospitalization status. **Results**: Comparisons between the two groups revealed that median SAA values were significantly higher in children with COVID-19 than in their healthy peers (21.45vs3.05 mg/L, *P*=0.002). There was no significant difference in the median serum SAA levels between mild and moderate/severe clinical disease (*P*=0.837). The SAA difference between the two groups with regards to hospitalization was not statistically significant (*P*=0.098). **Conclusions**: Although SAA level was found to be higher in children with COVID 19 compared to healthy controls, the sensitivity of SAA for the disease was found to be low. In addition, there was no difference between the groups in terms of clinical severity.

Keywords: *Cytokines, Diagnosis, Management, Outcome.*

Many biochemical markers have been investigated in the diagnosis and clinical severity in patients with coronavirus disease 2019 (COVID-19), including C-reactive protein (CRP), procalcitonin, lymphocyte count, and others [1-4]. Serum amyloid A (SAA) is an acute-phase protein mainly produced by the liver in response to proinflammatory cytokines secreted by the activated monocytes. This protein serves an essential role in inflammation and relates to the severity of inflammation. Currently, there are few reports about the relationship between SAA and COVID-19 [1]. Previous studies indicated that SAA plays essential roles in inflammatory diseases [5,6]. This study aimed to investigate the role of SAA in the diagnosis and clinical severity of COVID-19 in children.

METHODS

This study was carried out in our hospital after getting approval from the ethics committee of the pediatric infectious diseases department. Data of children diagnosed with laboratory-confirmed COVID-19 between March, 2020 and March, 2021 was retrieved. Patients with multisystem inflammatory syndrome in children (MIS-C) who tested positive for COVID-19, and those with missing details were excluded from the study. Serum samples were collected from COVID-19 positive patients attending outpatient clinics. The control group consisted of age- and gender-matched healthy individuals. Although reference values were available, comparisons were made with age- and gender-matched-healthy controls admitted in the same period.

Combined nasopharyngeal and or pharyngeal swab samples were taken from children with suspected COVID-19 and sent to medical microbiology laboratory of our hospital. Severe acute respirtory syndrome coronvirus 2 (SARS-CoV-2) was detected using reverse transcriptase-polymerase chain reaction (RT-PCR) (Bio-Speedy SARS-CoV-2 double gene RT-qPCR kit). Blood samples were collected between 8 AM and 12 PM. Serum samples were stored at –80 ºC after portioning. After all samples were collected, analysis was performed in batches. Serum amyloid A (SAA) levels were studied by nephelometric method in a BN II brand nephelometry device (Siemens Helathineers).

Demographic details, clinical symptoms, underlying diseases were also recorded, along with the laboratory reports. The guidelines of the CDC (Centers for Disease Control and Prevention) were used for classifications of COVID-19 severity into five categories according to the severity of the disease [7]. Asymptomatic patients were not included in our study. Disease severity was evaluated in two groups as mild and moderate severe.

Statistical analysis: Statistical analyses were performed by using SPSS software version 24.0 (IBM Corp). Descriptive statistics were used to summarize the patients' baseline characteristics, depending on the normality of distribution. Comparisons between groups for categorical variables were made by using the Chi square test. For two group comparisons of independent variables, the Student *t* test was used as a parametric test, whereas the Mann–Whitney *U* test was used as a non-parametric test. Receiver operating characteristic (ROC) curve analyses were conducted to determine diagnostic cut-off values and their sensitivity, specificity, and area under curve (AUC) values. Statistical significance was defined as *P* values less than 0.05.

RESULTS

A total of 80 children (40 COVID-19 positive; 40 healthy controls) were evaluated. Fever (57.5%), and cough (27.5%) were the most common presenting symptoms of the COVID-19 infected children. The demographic and clinical findings of children with COVID-19 is shown in **Table I**. Hospitalization rate was 42.5% (*n*=17), and 35.2% (*n*=6) of hospitalized cases were categorized into moderate/severe group, six (15%) patients had comorbidities. None were in the critical illness category. Three (50%) of the patients with underlying diseases were hospitalized and followed up in the moderate/severe group. The median (IQR) SAA values in COVID-19 group were significantly higher than that in healthy individuals [21.45 (5.94-105.4) vs 3.05 (1.6- 6.52) mg/L; *P*=0.002]. When the groups were compared according to disease severity, a significant difference was not found between mild disease and moderate/severe disease in terms of SAA levels (*P*=0.844). Comparison of the clinical and laboratory findings between mild disease and moderate/severe disease groups is shown in **Table II**. The serum SAA levels were similar across all categories of clinical severity. While neutrophil - lymphocyte ratio (NLR) was significantly higher in the moderate/severe group than mild group, all other inflammatory markers were similar in both categories of severity.

Comparison of the clinical and laboratory findings according to hospitalization status in patients with COVID-19 (**Web Table I)**, showed that there was no significant difference in SAA levels between the two groups. Other markers of inflammation were significantly higher in hospitalized patients.

The calculated cutoff value for SAA to identify COVID-19 patients was 5.82 mg/L. ROC curve for SAA values in COVID-19 patients vs healthy controls is shown in **Fig. 1** [AUC (95 % CI) 0.80 (0.70 -0.90); *P*<0.001].

DISCUSSION

Several biochemical parameters have been assessed for early diagnosis of disease, confirming and classifying disease severity, decision for hospitalization, and ICU admission [8,9]. SAA is one of the investigated parameters. The exact mechanism of increased SAA concentration in patients with COVID-19 is unknown. The differences in SAA levels between patients with COVID-19 and healthy participants observed in our study suggest that this biomarker may be particularly useful for identifying patients with COVID-19. However, SAA is not specific to COVID-19 and may be elevated in other respiratory diseases. In a similar study including adult patients in the literature, SAA was found to be higher than the healthy group [10]. In the results of our study, 80% sensitivity and 70% specificity are low for the diagnosing test. The reason for the low

Table I Demographic and Clinical Characteritics of Children With COVID-19 Enrolled in the Study (*N*=40)

Characteristics	*Value*
Male	18 (45)
Age (mo)[a]	156 (60-180)
Underlying medical conditions	6 (15)
Hospitalization	17 (42.5)
Symptoms and signs	
Fever	23 (57.5)
Cough	11 (27.5)
Runny nose	10 (25)
Weakness	8 (20)
Sore throat	7 (17.5)
Headache	6 (15)
Vomiting/nausea	6 (15)
Diarrhea	6 (15)
Abdominal pain	5 (12.5)
Laboratory tests	
WBC (x10³/uL)[a]	5700 (4300-10025)
ANC (x10³/uL)[a]	2750 (1750-5775)
ALC (x10³/uL)[a]	1850 (1300-2575)
N/L[a]	1.74 (1.06-2.84)
Platelets (x10³/uL)[b]	252800 (195750-291750)
Hemoglobin (g/dL)[a]	13.1 (12.12-14.05)
D-dimer (µg/L FEU)[b]	589.17 (300-632)
CRP (mg/L)[a]	4.35 (1.32-33)
Procalcitonin (µg/L)[a]	0.04 (0.012-0.08)
SAA (mg/L)[a]	21.45 (5.94-105.4)
Clinical severity	
Mild	34 (85)
Moderate and severe	6 (15)

Values in no. (%),[a]median (IQR) or [b]mean (SD). WBC:white blood cell; ANC:absolute neutrophil count; ALC:absolute lymphocyte count; N/L:neutrophil lymphocyte range; CRP:C-reactive protein; SAA:serum amyloid A.

Table II Clinical and Laboratory Findings in Children With Varying Severity of COVID-19 (*N*=40)

Characteristics	*Mild (n=34)*	*Moderate and severe (n=6)*
Clinical charateristics		
Male	16 (47.0)	2 (33.3)
Age (mo)[a]	156 (60-180)	109 (25-180)
Underlying medical conditions	6 (17.6)	0
Hospitalization	11 (32.3)	6 (100)
Fever	19 (55.9)	4 (66.6)
Cough	6 (7.6)	5 (83.3)
Runny nose/sore throat	9 (26.5)	1 (16.6)
Weakness	8 (23.5)	0
Laboratory characteristics		
WBC ($x10^9$/L)[a]	5.7 (4.3-9.2)	7.65 (4.1-10.5)
ANC ($x10^9$/L)[a]	2.65 (1.7-5.4)	5.15 (2.1-8.2)
ALC ($x10^9$/L)[a]	1.9 (1.3-2.6)	1.4 (1.1-2.4)
N/L range[a]	1.39 (1.02-2.64)	4.18 (1.9-6.3)
CRP (mg/L)[a]	4.25 (0.1-157.5)	18.2 (2.1-35.2)
Procalcitonin (µg/L)[a]	0.04 (0.01-0.08)	0.07 (0.04-0.09)
SAA (mg/L)[a]	18.4 (5.94-97.6)	55.9 (18.5-117)

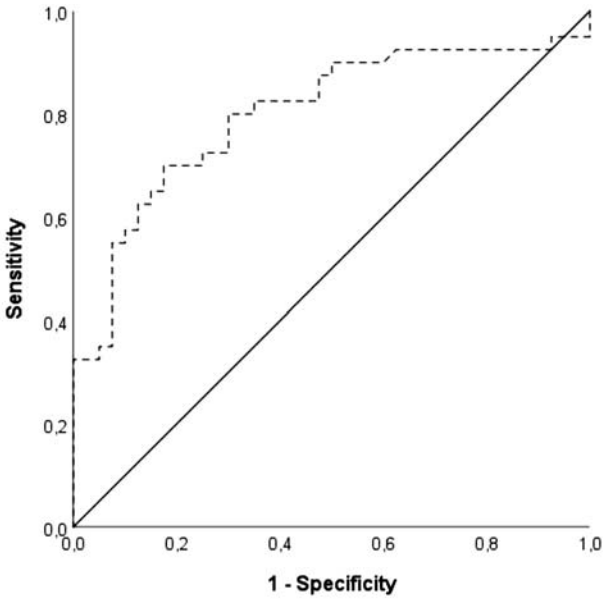
Values in no. (%) or [a]median (IQR). WBC:white blood cell; ANC: absolute neutrophil count; ALC:absolute lymphocyte count; N/L: neutrophil lymphocyte range; CRP:C-reactive protein; SAA: serum amyloid A.

sensitivity and specificity may be attributed to the small number of cases in our study. Investigation of the diagnostic value of SAA in the diagnosis of COVID 19 in children with studies involving more participants may reveal more clearer results.

In the literature, it has been reported that SAA can be one of the biomarkers for monitoring and it has a definite value in predicting the prognosis [11]. Previously, based on the data from 132 COVID-19 patients, Li, et al. [2] have reported that SAA/lymphocyte count, CRP, SAA, and lymphocyte count were valuable to evaluate the disease severity. In a meta-analysis examining adult patient studies, it was reported that NLR and SAA values were associated with disease severity [12]. A meta-analysis data shows that; while SAA was significant in showing the severity of the disease according to CRP, it was not sufficient to differentiate it from non-infected cases [13]. In a study that included only 13 severe patients, the variability of SAA according to disease severity was emphasized [14]. However, in our study, SAA was similar in groups in terms of clinical severity and hospitalization. Since our study is one of the limited number of publications on pediatric patients, studies involving larger participants are needed. Although SAA could not be helpful in terms of prognosis in our study, its higher detection rate in hospitalized patients may be an indirect guide about the severity of the disease.

Elevated levels of CRP, procalcitonin, D-dimer and low lymphocyte count have been associated with severe forms of COVID-19 as well as high NLR [10]. Our results indicated that WBC, ANC, absolutely lymphocyte count (ALC), CRP, platelets, and procalcitonin were not significantly different between the moderate/severe group and mild group. NLR was significantly higher in the moderate /severe group. Lymphocyte counts of the two groups were lower than the normal range and but not markedly different. A systemic review report compared the ALC of the severe and mild group and it concluded that lymphocytopenia is associated with in the risk of the COVID-19 severity [15].Our findings are consistent with other studies.

This study has several limitations. First, it was performed in a specific setting and only a relatively small number of children who were admitted to our center were enrolled. This prevents further generalization of the findings. This may also pose a bias in selection of the patients with COVID-19. Secondly, serum sampling of the children with COVID-19 was not based on the timing of different phases of the course of the SARS-CoV-2 infec-



AUC	Sensitivity	Specifity	PPV	NPV
0.800	80	70	72.7	77.7

SAA:serum amyloid A; AUC:area under the curve; PPV:positive predictive value; NPV:negative predictive value.

Fig. 1 ROC curve for serum amyloid A value in children with COVID-19 and healthy contols for a cutoff value of 5.82 mg/L.

WHAT THIS STUDY ADDS?

- Serum amyloid A levels did not differentiate between severity categories of coronavirus disease 2019 (COVID-19) in children.

tion. We enrolled healthy children as a control group, but it would have been more appropriate to choose patients with other respiratory tract infections. The low incidence of other respiratory tract infections and the increase in the frequency of admission findings other than respiratory tract infections led us to prefer healthy controls.

Larger studies of SAA in pediatric COVID-19 patients are needed, as most of the previous work was from adult patients. However, since the number of patients with moderate/severe clinical severity is low, this relationship can be revealed more clearly with studies with more extensive participation.

Ethics clearance: Provide name of IEC. Institutional Ethics Committee; Permission No. Provide, dated March 29, 2021.
Contributors credit: ST: conceptualizing, writing, and critically reviewing the article; EKO: statistical analysis, article writing; AET: data collection. AS: data collection. GÜ: data collection; YEK: data collection; SO: hormonal measurements; BIB: hormonal measurements; AKA: conceptualizing, writing and critically reviewing the manuscript; DYÇ: conceptualization, writing and critical review of the manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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