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Investigation of serum neopterin levels and adenosine deaminase enzymatic activity in measles infection

Abstract. The aim of this study was to investigate the serum neopterin level and ADA activity in acute measles infection and determine whether there is a correlation between the measles optical density values, and the neopterin and ADA levels. The neopterin level and ADA activities were investigated in the samples of 136 measles IgM-positive patients along with those of 40 measles IgM-negative patients as the control group. The most important findings of the study were the determination of significantly higher neopterin levels and ADA activity in the measles IgM-positive group when compared to the measles IgM-negative group. The high neopterin level and ADA activity in measles before IgM becomes positive in some of the patients in the first 3 days suggested that they can be used as a preliminary and supportive marker for the preliminary diagnosis of measles. These findings have shown that the neopterin level and ADA activity can assist in the diagnosis of measles in the acute period as biomarkers.
Key words: Measles, neopterin, adenosine deaminase, biomarkers, ELISA.

Introduction

Neopterin is used as a trendy biological marker in recent years in severe conditions such as in transplantation, inflammatory diseases, and autoimmune and malignant diseases, where the cellular immune system is activated [1]. Cytokines are an indicator of proinflammatory immune state delivered by human monocytes and macrophages upon stimulation by interferon-gamma [2-6]. Neopterin may be a prognostic determinant in the early stages of the disease [7, 8]. Neopterin also increases in conditions that accompany the increase of endogenous interferons, such as viral infections (especially HIV), infection of intracellular pathogens (tuberculosis, malaria, etc.), autoimmune diseases, inflammatory diseases, allograft rejection, malignant diseases, and hereditary pteridine metabolism. In studies conducted, has IFN-leds to neopterin production and release in human monocytes and macrophages *in vitro* [1, 8, 9].

Numerous clinical and experimental studies have demonstrated the relationship between neopterin production and cellular immune activation, and a strong link between neopterin levels and the severity and progression of infectious and inflammatory diseases has been demonstrated [10, 11]. Adenosine deaminase (ADA) is an enzyme required for purine metabolism that plays an important role in the differentiation of lymphoid cells. ADA deficiency causes autosomal recessive diffuse combined immunodeficiency disease with impaired cellular immunity, B- and T-lymphocyte dysfunction, and decreased immunoglobulin production [12]. ADA deficiency progresses with lymphopenia, severely impaired cellular and humoral immunity, growth retardation, and serious fatal infections [12, 13]. Measles (Rubeola, Measles, Morbili) is an acute viral disease of childhood with rash [14]. Humoral and cellular immunity occurs in those who suffer from the disease. Immunity after natural infection is thought to be lifelong [15]. In immunocompromised patients, measles may have a longer, more severe, and fatal course, and the severity of the disease depends on the severity of the cellular immunity disorder. Antibody formation is considered in the diagnosis of the disease, but immunity is mainly dependent on T-lymphocyte functions and memory [15]. The purpose of the current study was to 1) investigate the serum neopterin level and ADA activity in acute measles infection; 2) determine whether there is a correlation between measles optical density (OD) values, and neopterin and ADA levels; 3) to examine the values between the acute phase of the disease and

the recovery period; and 4) to compare the results with the control group and examine the relationship of the obtained data with the demographic findings.

Materials and methods

Serum samples of a total of 176 patients, comprising 102 women and 74 men, were included in the study. During the measles epidemic in Turkey in 2014–2015, serum samples that fit the case definition of measles and were confirmed by the laboratory were used in the current study. A pool of 176 samples, from 136 IgM-positive measles patients and 40 measles IgM-negative patients (control group), were created from the samples separated at the National Virology Reference Laboratory of the General Directorate of Public Health and stored at –80 (±10) °C. The neopterin level and ADA activity were investigated in all of the samples. The micro ELISA method (Neopterin ELISA, IBL, Germany), and ELISA washer and reader devices were used for the neopterin test. ADA analysis was performed spectrophotometrically using a Shimadzu UVmini 1240 spectrophotometer (Kyoto, Japan) following the method described by Giusti. The Enzygnost Anti-Measles Virus/IgM (Siemens, Marburg, Germany) kit was used for the determination of the measles IgM antibodies. The method was based on the indirect ELISA principle, and the test study was conducted in accordance with the instructions in the package insert of the commercial kit used.

Statistical analysis. SPSS Statistics for Windows 15.0 (SPSS Inc., Chicago, IL, USA) was used to evaluate the data obtained from the study and create tables. The mean, standard deviation, median, minimum, and maximum values were used for the presentation of the continuous variables (quantitative variables) obtained by the measurements, and the frequency and percentage values were used for presentation of the categorical variables (qualitative variables). The Fisher Exact test was used to evaluate the categorical variables. In the comparison of the quantitative variables, whether the parametric test conditions (investigation of conformity to normal distribution) were achieved was investigated using the Kolmogorov-Smirnov or Shapiro-Wilk test. The Mann-Whitney U test was used to compare 2 independent groups, as the parametric test conditions were not met. The Spearman correlation coefficient was used to examine the relationships between the variables and the relationships were also shown as a scatter plot. In all of the statistical analyses, statistical significance was accepted as $P < 0.05$.

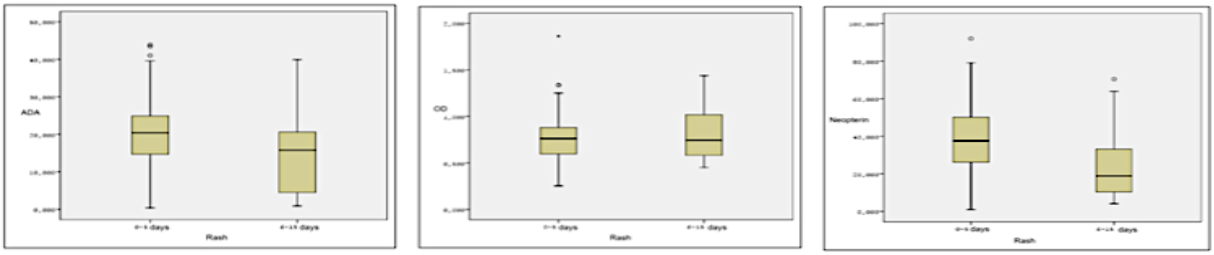
Results and discussion

A significant difference was found between the OD, neopterin and ADA levels in the IgM-positive and IgM-negative measles groups, and the results are summarized. Of the samples that exhibited positive results, 86.8% consisted of blood samples taken within the first 0 to 5 days from the date of rash onset, and 13.2% were taken between days 6 and 15. All of the samples that exhibited negative results consisted of blood samples taken in between days 0 and 5. In the measles IgM-positive group, there were no statistically significant differences between days 0 and 5, and days 6 and 15 in terms of the measles IgM OD values with regards to the date of rash onset ($P = 0.579$). In the measles IgM-positive group, the median neopterin level between days 0 and 5 with regards to the date of rash onset was 37.58, and the median value between days 6 and 15 was 18.83. There was a statistically significant difference between days 0 and 5, and days 6 and 15 ($P = 0.002$). In the measles IgM positive group, according to the date of the rash, ADA values were found to be higher in the 0-5-day period, but there was no statistically significant difference between the 6-15-days period ($P = 0.082$) (Figure 1).

When the OD values between days 0 and 5 in the measles IgM-positive and IgM-negative groups were compared, it was determined that the measles IgM OD values were higher between days 0 and 5, and a statistically significant difference was found between them. The neopterin levels and ADA values were found to be higher between days 0 and 5 in the measles IgM-positive group, and a statistically significant difference was found when compared to the measles IgM-negative group ($P < 0.001$ for all) (Figure 2).

In the measles IgM-positive group, the median neopterin level value was found to be higher in the males, and a statistically significant difference was found between the males and the females, ($P = 0.032$). No statistically significant difference was found between the males and the females in terms of the ADA activity ($P = 0.807$). There were no statistically significant differences between the males and females in the measles IgM-negative group in terms of the neopterin level and ADA activity ($P = 0.871$ and $P = 0.705$). In the measles IgM-positive group, there was no statistically significant correlation between the measles IgM OD and neopterin levels with regards to age. The P-values were calculated as 0.068 and 0.640, respectively. There was a significant negative correlation between ADA and age ($P = 0.008$). In the

measles IgM-negative group, there was no statistically significant correlation between the measles IgM OD and ADA values with regards to age. The P-values were calculated as 0.833 and 0.542, respectively. There was a significant negative correlation between the neopterin level and age (P = 0.019) (Figure 3).



ADA: adenosine deaminase

OD: Optical density

Figure 1. Box-plot graph of the OD value, neopterin levels, and ADA activity in the IgM-positive measles groups with regards to the date of rash onset.

Figure 1 – Box-plot graph of the OD value, neopterin levels, and ADA activity in the IgM-positive measles groups with regards to the date of rash onset, where: ADA – adenosine deaminase; OD – Optical density

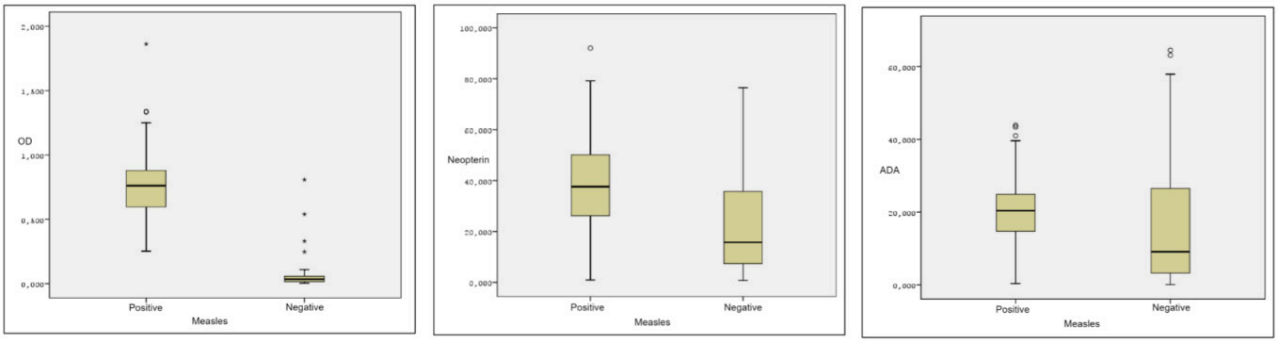


Figure 2 – Box-plot graph of the OD values, neopterin levels, and ADA activity between days 0 and 5 in the IgM-positive and IgM-negative measles groups, where: ADA – adenosine deaminase; OD – Optical density

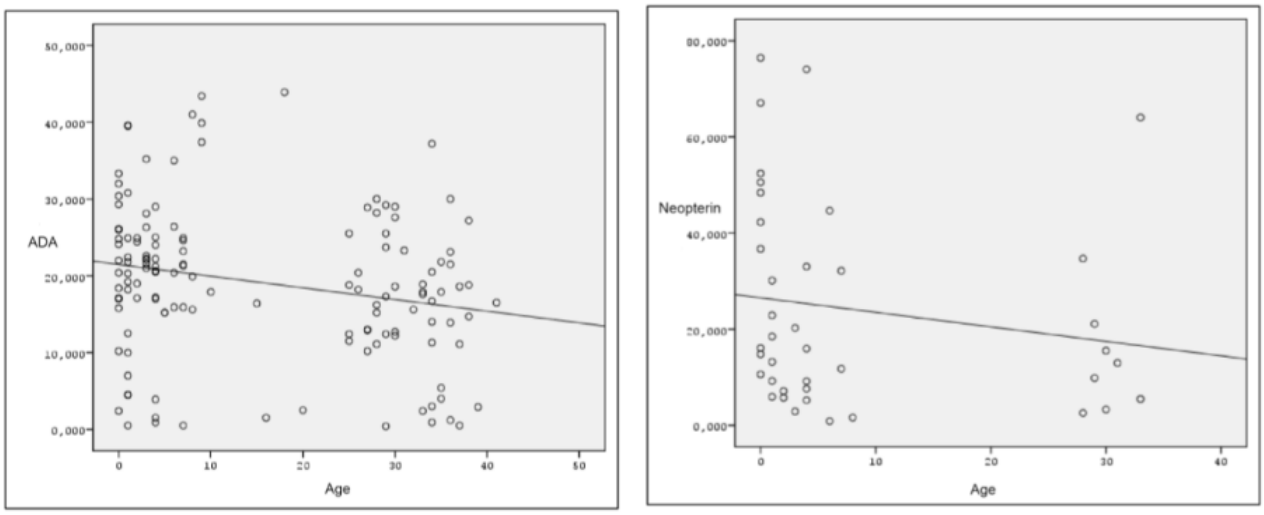


Figure 3 – Plot of the negative correlation between the ADA activity and age in the IgM-positive measles group

Neopterin is considered to be a biochemical marker of stimulated cellular immune response [1]. It is known that increased neopterin levels occur in viral infection. The literature comprises very old studies of measles in patients with subacute sclerosing panencephalitis (SSPE); however, there have been no follow-up studies thus far, regarding neopterin levels and ADA activity in patients suffering from acute measles infection [16, 17]. Neopterin may actually be a visible early diagnosis marker. Recommendations have been made that neopterin should be useful as a prognostic marker in a retrospective study for HIV positivity [18]. In studies conducted on patients with tuberculosis, serum neopterin levels were reported to be higher than the control group [19]. In another study, it was stated that increased neopterin levels were associated with mortality [20]. In the current study, the measles IgM OD values were expressed as minimum, median, and maximum values in the IgM-positive measles group, respectively, as 0.25, 0.76, and 1.86 (Table 1).

Table 1 – OD value, and neopterin and ADA levels in the IgM-positive and IgM-negative measles groups

		OD					Z	P
		Mean	Standard deviation	Median	Minimum	Maximum		
Measles	Positive	0.77	0.25	0.76	0.25	1.86	-9.21	<0.001
	Negative	0.08	0.15	0.03	0.01	0.10		
		Neopterin					Z	P
		Mean	Standard deviation	Median	Minimum	Maximum		
Measles	Positive	36.48	18.77	35.11	0.94	92.02	-3.77	<0.001
	Negative	23.92	21.48	15.73	0.86	76.45		
		ADA					Z	P
		Mean	Standard deviation	Median	Minimum	Maximum		
Measles	Positive	19.13	9.86	19.10	0.40	43.90	-2.54	0.011
	Negative	16.94	18.77	9.10	0.10	64.50		

Since an OD value above 0.20 is considered positive, this indicated that the study group included a large patient sample with low-positive, positive, and high-positive values. Significantly higher neopterin levels were observed in the measles IgM-positive group when compared to the measles IgM-negative group, which was one of the main objectives of this study, and emerged as one of the most important findings of the study ($P < 0.001$). Although there are no studies in the literature regarding neopterin levels in acute measles infection, similar results were obtained in studies investigating neopterin levels in other infectious diseases. [21]. Reported that serum neopterin levels in patients with chronic kidney disease were significantly correlated when inflammatory markers such as hsCRP, IL-6, and IFN-were increased [21]. In our study, the neopterin level measured in the measles IgM positive group was found to be significantly higher than in the negative group ($p <0.001$). This supported the idea that neopterin can be used as a biomarker in the early diagnosis of measles. When analyzed according to the date of rash onset, it was observed that the neopterin levels were found to be significantly higher in the blood samples taken in the first 5 days when compared to those taken between days 6 and 15 ($P = 0.002$). In measles patients, specific IgM antibodies cannot be detected in 20% of the patients in the first 3 days, and the presence of a virus can only be detected by molecular tests. For this reason, high levels of neopterin and ADA in the first 5 days can be helpful in the diagnosis of measles in the acute period. In a study conducted on acute respiratory diseases, neopterin levels in the early period and the recovery period were compared, and the mean neopterin levels were determined as 34.2 nmol/L in the acute serum of the patients and 5.1 nmol/L in the healing sera [22]. This showed that neopterin levels can be helpful in diagnosis, especially in the first days of the disease and in the acute phase. In the measles IgM-positive group herein, no statistically significant correlation was found between the neopterin levels and age ($P = 0.64$). In the IgM-negative measles group, a significant negative correlation was found

between the neopterin levels and age (P = 0.019). In other words, it was observed that the younger the age, the higher the neopterin level. In another study conducted, it was shown that serum neopterin levels changed with age, without any association with any disease [22]. Neopterin levels were found to be high in the serum of patients under the age of 18 and above the age of 75, while the age-related neopterin levels did not change in patients between 18 and 75 years of age [23, 24]. In a study conducted by Daito et al. on 14 patients with chronic hepatitis B, where 8 patients were over 30 years of age and 6 were under 30 years of age, no significant relationship was found between the serum neopterin levels and age [24]. In the study of Lucas et al., it was shown that serum neopterin levels increased significantly as the age of the patients increased, in 302 healthy adults, without infectious disease, under stress [25]. The reason for such a correlation in the negative patient group was thought to be due to the fact that this group of patients had a fever with a non-measles rash or that the median age value of the negative patient group was lower than that of the positive group. Considering that the group of patients with measles IgM-negative was actually the group of patients with a fever, rash complaints, and whose samples were sent with a pre-diagnosis of measles, the lower neopterin levels in this group suggested that neopterin can also be used in the differential diagnosis of other rash and fever agents in the acute phase of measles.

ADA is recognized as a nonspecific marker of T-lymphocyte activation and cellular immunity [26]. It is known that ADA activity increases in infectious diseases, such as tuberculosis, in which T-lymphocytes play an important role [27-29]. In the present study, higher median values were obtained in the measles IgM-positive group when compared to the measles IgM-negative group in terms of ADA activity, and the difference was found to be statistically significant (P = 0.011). There are no studies in the literature regarding ADA activity in acute measles infection. There are studies reporting increased pleural fluid ADA activity in patients with TB pleurisy [30, 31]. In a study by Solomon et al., the pleural fluid levels and serum ADA activity were investigated, and it was found that serum ADA activity was higher in patients with TB than in those with malignancy, pneumonia, and rheumatoid arthritis [32]. Herein, a negative correlation was found between age and ADA activity in the measles IgM-positive group, and it was found to be statistically significant (P = 0.008). No significant correlation was found in the measles IgM-negative group. In a study of Kaya et al., 73 hepatitis B and 71 hepatitis C patients were examined, and serum the ADA and transaminase activity was found to be significantly higher than in the control group [33]. One of the limitations of this study was that the patients could not be followed-up in terms of the prognosis of the disease, mortality and morbidity, development of complications, such as SSPE, etc. Another limitation of this study was that vaccination information could not be obtained for all of the patients; hence, no comparison could be made between IgM positivity due to vaccine and IgM positivity due to wild virus in terms of the neopterin levels and ADA activity. The median neopterin value was found to be higher in the men in the measles IgM-positive group, and a statistically significant difference was found between females and the males (P = 0.032). Although a clear explanation could not be made regarding the reason for this, different results were obtained in different studies depending on the population in which the study was conducted [2, 29, 30, 34].

Conclusion

Significantly higher neopterin levels and ADA activity in the measles IgM-positive group when compared to the measles IgM-negative group emerged as one of the most important findings of this study. When analyzed according to the date of rash onset, it was observed that the neopterin levels and ADA activity were found to be significantly higher in blood samples taken during the first 5 days when compared to those taken between days 6 and 15. With these findings, both parameters were found to be higher in the acute period when compared to the recovery period, suggesting that they behaved like an acute phase reactant. The high neopterin level and ADA activity in measles before IgM becomes positive in some of the patients in the first 3 days suggested that they can be used as a preliminary and supportive marker for the preliminary diagnosis of measles. In conclusion, the findings herein showed that the neopterin level and ADA activity, as biomarkers, can help diagnose measles in the acute phase.

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