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Evaluation of serum adenosine deaminase activity and autoantibodies in systemic lupus erythematosus

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is a complex autoimmune disease with no single specific and sensitive test available for its diagnosis. Adenosine deaminase (ADA) is an enzyme that can act as an indicator of cellular immunity reflecting the extent of inflammation. The aim of this study was to investigate the role of serum adenosine deaminase activity and its association with antinuclear antibodies (ANA) and anti-dsDNA (anti-double stranded deoxyribonucleic acid) in SLE patients.

Material and methods: In this study, we included 36 diagnosed case of SLE as per the American Rheumatology Association (ARA) criteria and 30 healthy control. Serum ADA activity was measured by a spectrophotometric technique based on Giusti and Gallanti. ANA and Anti ds-DNA were measured by indirect Enzyme-Linked Immune Sorbent Assay (ELISA). Normal values of serum ADA activity, ANA and anti ds-DNA was < 25 IU/L, <1 0 U/mL and <25 IU/mL respectively.

Results: The mean ADA activity (37.03 ± 13.03 IU/L) in SLE patients was higher as compared to control (18.23 ± 10.12 IU/L), and the difference was statistically significant ($p < 0.001$). Median values of ANA and anti-dsDNA in SLE patients was 16.9 (8.5, 42.8) and 24.7 (20.5, 45.0) respectively. Serum ANA was positive in 25 out of 36 cases, anti-dsDNA was positive in 17 out of 36 cases and ADA was positive in 29 out of 36 cases. We observed a weak positive correlation between ANA and anti-dsDNA ($r = 0.46$, $p = 0.005$), whereas a moderate correlation between ANA and ADA ($r = 0.525$, $p = 0.001$) in SLE patients.

Conclusion: Serum ADA was significantly higher in SLE patients as compared to control and more specific than autoantibodies. Determination of ADA activity is a reliable, cost-effective, and easy test that can be used as an alternative parameter for diagnosing and evaluating disease activity in SLE patients.

Key words: SLE, ADA, ANA, anti-dsDNA

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Introduction

Adenosine deaminase is a hydrolytic enzyme that catalyzes the conversion of adenosine or 2'-deoxyadenosine to 2'-deoxyadenosine and is widely distributed in many tissues [1]. The role of ADA has been widely discussed and its role in regulating immune response has been demonstrated experimentally. Numerous evidence highlights its role in the function, maturation and regulation of immunological responses [2–5]. Impaired immune tolerance is an important characteristic of autoimmune diseases, which led many researchers to investigate the role of ADA in autoimmune disease [6, 7]. Several studies have shown increased ADA activity in diseases like rheumatoid arthritis (RA), and SLE [8–11]. Autoimmune disease can

be organ-specific or non-organ-specific, in which the body damages self-tissues [12]. SLE is a connective tissue disease of idiopathic origin, the spectrum of which covers a wide array of clinical and laboratory manifestations. While the etiology of SLE is thought to be multifactorial, the disease is characterized by the production of autoantibodies. SLE is a prototypic example of non-organ specific autoimmune disorder in which plethora of autoantibodies is formed especially against nuclear DNA. Moreover, T-cell/B-cell dysregulation are an important hallmark of SLE [13, 14]. Though the 5-year survival rate is 90% [15], however, survival rate has not improved since 1980. Due to its multi-organ involvement of SLE no single specific test is available till date in making its diagnosis. Hence it becomes crucial to look for a test that can be more specific and sensitive

in diagnosis and monitoring disease activity. Therefore this study was undertaken to assess the role of serum ADA level in SLE patients and explore its role in the diagnosis of the same.

Material and methods

Study population and setting

This was a hospital-based cross-sectional study conducted in the Department of Biochemistry in collaboration with the Department of Dermatology and Venereology. A total of 36 diagnosed cases of SLE patients and 30 age and sex-matched healthy control were included in the study. A brief interview was taken from the patient and control group to fill up the preformed proforma.

Ethical clearance

The research proposal was approved by the Institutional Review Committee, of B.P. Koirala Institute of Health Sciences, Dharan, Nepal and was conducted in accordance with the Declaration of Helsinki adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964. The study was initiated only after taking the written informed consent from the participants.

Sample collection and analysis

Three mL venous blood was drawn in a plain vial following the standard protocol. The samples were collected in the Immunoassay Laboratory and the serum was separated after centrifuging the sample for 10 minutes at 3000 rpm. Collected serum was used for the analysis of ADA, ANA, anti-dsDNA and other biochemical parameters

Analytical procedures

Serum ADA activity: The ADA activity was measured at 37°C according to the method of Giusti and Galanti, based on the Bertholet reaction, i.e. the formation of colored indophenol complexes from ammonia liberated from adenosine, and quantified spectrophotometrically at 625nm. ANA and anti-dsDNA were measured by indirect Enzyme-Linked Immunosorbent Assay (ELISA). As per our Lab, normal values of serum ADA activity, ANA and anti-dsDNA were < 25 IU/L, < 10 U/mL and < 25 IU/mL respectively.

Statistical analysis

The values are expressed as mean \pm SD, median and interquartile range for continuous variable and number and percentage for nominal variables. Normality of

the data was tested by the Kolmogorov Smirnov test. Statistical analysis of differences within and between the study groups was carried out using the Student t-test and Chi-square test. Pearson's correlation coefficients were used to determine the relationships between parameters. The analysis was done by Statistical Package for the Social Sciences (SPSS) 16.0 version software. $P < 0.05$ was considered as statistically significant.

Results

In this study, we included 36 diagnosed cases of SLE which were referred to the Biochemistry Laboratory from the Dermatology department. Thirty age and sex-matched healthy control were also enrolled in the study. Mean age of SLE patients was 34 ± 13.49 years whereas that of control was 36.23 ± 15.34 years and was not statistically significant which fulfilled the age-matched criteria for selecting the two groups. There were 86% of females in SLE patients and 80% female in the control group and the difference was not significant. We did not observe any significant difference in BMI between the groups ($p = 0.062$). CRP level was quite high (12.8 ± 12.2 mg/dL) in SLE group as compare to the control (3 ± 2.8 mg/dl) and was statistically significant ($p = 0.02$). No significant difference was seen in Hemoglobin and ESR level among the two groups. Serum ADA activity in SLE group (37.03 ± 13.03 IU/L) was higher as compared to control (18.35 ± 7.71 IU/L) and the difference was statistically significant ($p < 0.001$). The median ANA and anti-dsDNA in the SLE group was 16.9 (8.5, 42.8) and 24.7 (20.5, 45.0), respectively. Serum ANA was positive in 25 out of 36 cases, anti-dsDNA was positive in 17 out of 36 cases whereas ADA was positive in 29 out of 36 cases. A weak positive correlation between ADA and anti-dsDNA ($r = 0.46$, $p = 0.005$) was observed whereas a moderate correlation between ADA and ANA ($r = 0.525$, $p = 0.001$) was observed in SLE patients (Fig. 1, 2).

Discussion

The exact pathophysiology of SLE is not known, although many etiologies like infection, genetic, immunologic etc., have been described [16]. In this study, we investigated the ADA activity in SLE patients and found that its level was significantly high as compared to healthy control. Previous studies have also reported increase serum ADA activity in various diseases, including infection, malignancies and liver disease [17]. Various studies in the past have reported high ADA activity in SLE patients [10, 11, 18], our results are also in accordance with them. Even though the mechanism by which ADA activity increases has not been fully elucidated,

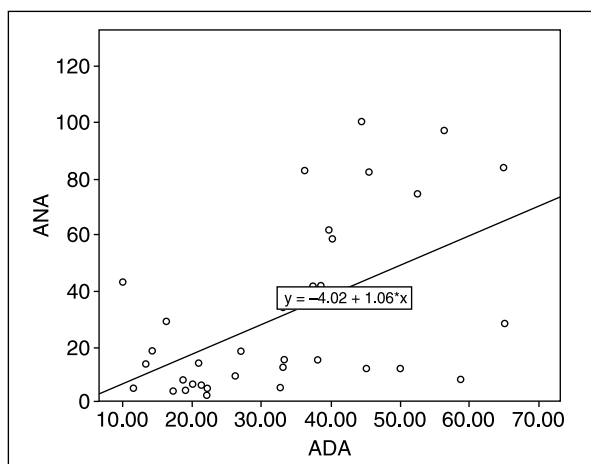


Figure 1. Correlation between adenosine deaminase and antinuclear antibodies in systemic lupus erythematosus patients

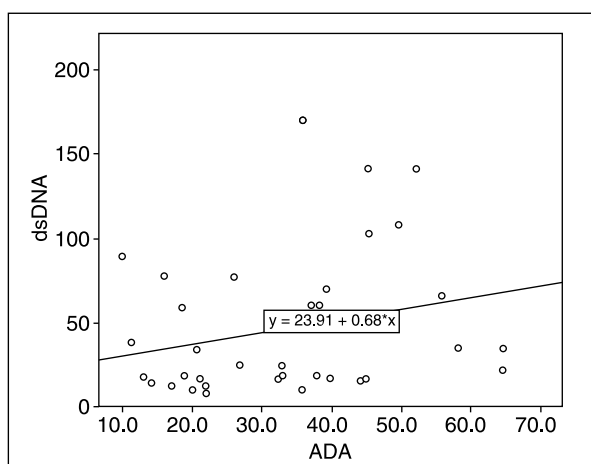


Figure 2. Correlation between adenosine deaminase and anti-double-stranded deoxyribonucleic acid in systemic lupus erythematosus patients

possibly its increase in SLE patients can be correlated to altered immune response [19, 20].

As per literature review female are more prone to autoimmune diseases as compared to male. A similar finding was observed in our study as well, where 86% of the SLE patients were female. Laxminarayana *et al.* [21] have reported that female was having 6–10 fold higher chance of acquiring SLE compared to male.

At present, ANA and anti-dsDNA are an important parameters in the diagnosis of SLE, as per the American Rheumatology Association (ARA) criteria. Though many more criteria are included to complete the diagnosis. In this study serum, ANA and anti-dsDNA in SLE patients was also evaluated. We observed ANA was positive in 69% of the cases, anti-dsDNA was positive in only 47% of the cases whereas ADA was positive in 80% of the cases. This results highlight the utility of ADA in diagnosing SLE as it is more specific than the conventional antibodies.

Conclusion

This study concludes that serum ADA activity was higher in SLE patients as compared to control group and is more specific in diagnosing the SLE as compared to autoantibodies. Determination of ADA levels is a reliable, cost-effective and easy test that can be used as alternative parameters representing disease activity in SLE patients. However, it would be too early to conclude ADA as a marker of SLE, therefore, further studies with a large number of samples is required to generalize our findings.

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Table 1. Basic and laboratory parameter among the groups

Variables	SLE patients	Control	P value
Age (years)	34 ± 13.49	36.23 ± 15.34	0.590
Gender, n (%)			
Male	5 (13.8)	6 (20)	0.630
Female	31(86.2)	24 (80)	
BMI (kg/m ²)	26.2 ± 4.3	24.4 ± 3.5	0.063
Hb (g/dL)	11.3 ± 1.4	10.9 ± 0.6	0.06
ESR (mm/hr)	33.7 ± 23.5	28.1 ± 10.3	0.08
CRP (mg/L)	12.8 ± 12.2	3 ± 2.8	0.02
ADA (IU/L)	37.03 ± 13.03	18.35 ± 7.71	< 0.001
ANA (U/mL)	16.9 (8.5, 42.8)	—	NA
dsDNA (IU/L)	24.7(20.5,45.0)	—	NA

ADA — adenosine deaminase; ANA — antinuclear antibodies; BMI — body mass index; dsDNS — double-stranded deoxyribonucleic acid; SLE — systemic lupus erythematosus

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Conflict of interest

The authors report no competing interests.

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