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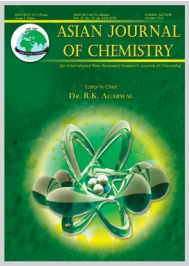

Effect of *Cutaneous Leishmaniasis* Treatment on Serum Adenosine/Deoxyadenosine Deaminase Activities

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Adenosine deaminase is an enzyme present in a great number of plants and animals, found from simple intervebrates to human beings. Adenosine deaminase and deoxyadenosine deaminase activities increase substantially during mitogenic and antigenic responses of lymphocytes and conversely lymphocytes blastogenesis. Various diseases characterized by the alteration of cell-mediated immunity can be effected by the serum activity of adenosine deaminase. *Cutaneous leishmania* is an intracellular parasite that targets and multiplies within phagocytic cells of the innate immune system such as the macrophage, dendritic cell and neutrophil. In this study, we investigated the role of treatment on adenosine deaminase and deoxyadenosine deaminase activities in the *cutaneous leishmaniasis* patients. For this aim, 25 treated *cutaneous leishmaniasis* patients, 19 untreated patients and 25 healthy control subjects were enrolled in this study. The clinical diagnosis was parasitologically confirmed Giemsa stain and by culture NNN medium. Adenosine deaminase and deoxyadenosine deaminase activities were measured spectrophotometrically by the method of Giusti. Serum adenosine deaminase and deoxyadenosine deaminase activities were higher in *cutaneous leishmaniasis* patients than the controls and also, adenosine deaminase and deoxyadenosine deaminase activities decreased in treated patients as compare with non-treated *cutaneous leishmaniasis* patients. According to these results, it is suggested that evaluation of adenosine deaminase activity in serum of patients with *cutaneous leishmaniasis* could be considered a useful monitoring tool for their infection status. Therefore, adenosine deaminase can be a predictive and sensitive parameter of *leishmaniasis* treatment.

Key Words: *Cutaneous leishmaniasis*, Adenosine deaminase, Deoxyadenosine deaminase.

INTRODUCTION

Leishmaniasis, a vector-borne disease caused by obligate intramacrophage protozoa, is characterized by diversity and complexity[1]. *Leishmaniasis* is endemic in areas of the tropics, subtropics and southern Europe, in settings ranging from rain forests in the Americas to deserts in western Asia and from rural to periurban areas[2]. Several clinical syndromes are subsumed under the term of *leishmaniasis*: most notably visceral, *Cutaneous* and mucosal *leishmaniasis*, which result from replication of the parasite in macrophages in the mononuclear phagocyte system, dermis and naso-oropharyngeal mucosa, respectively[3]. *Leishmaniasis* is an important public health problem, comprising zoonotic diseases caused by members of the genus *Leishmania* widely distributed in tropical and subtropical regions throughout the world[2,3].

Leishmania is an intracellular parasite that targets and multiplies within phagocytic cells of the innate immune system such as the macrophage, dendritic cell and neutrophil. Brisk replication can result in cell lysis followed by infection of surrounding macrophages. In a typical situation, infection and detection result in recruitment of inflammatory cells that in turn stimulate members of the adaptive immune response in the pathogenesis and healing of the *Cutaneous leishmaniasis*[1].

Adenosine deaminase (ADA) is an enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine to inosine and deoxyadenosine deaminase (DADA)[4-7]. Deoxyadenosine deaminase is synonym of adenosine deaminase enzyme and converts deoxyadenosine to deoxyinosine[7]. Adenosine deaminase is an enzyme present in great number of plants and animals, found from simple intervebrates to human beings and is widely distributed in lymphoid tissue, with the principal biological activity of adenosine deaminase being detected in T lymphocytes[1,8,9]. Its main physiological activity is related to lymphocytic proliferation and differentiation. The activity of this enzyme increases substantially during mitogenic and antigenic responses of lymphocytes and conversely lymphocytes blastogenesis[10,11].

Various diseases characterized by the alteration of cell-mediated immunity, such as rheumatoid arthritis, systemic lupus erythematosus and tuberculosis, are effected by the serum activity of adenosine deaminase, making adenosine deaminase a non-specific marker of cell-mediated immunity[12-17]. As a marker of cellular immunity, its plasma activity is found to be elevated in diseases in which there is a cell-mediated immune response[2,17-19]. High serum adenosine deaminase activities were observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, hepatoma, tuberculosis typhoid, infective mononucleosis and certain malignancies, especially those of hematopoietic origin[6,14].

It has been suggested that monocyte-macrophage cell system or lymphocytes contribute to changes in serum adenosine deaminase activity. The human immune system's ability to function properly is impaired by the deficiency of adenosine deaminase, resulting in severe combined immunodeficiency (SCID) characterized by severe T lymphocyte dysfunction and agammaglobulinemia[4,11-13,20]. It is known that serum adenosine deaminase activity increases both in visseral and *Cutaneous leishmania*[1,2]. However, the alteration of serum adenosine deaminase activity in *Cutaneous leishmania* is not known clearly before and after the treatment. The aim of this study is to investigate the role of treatment on adenosine deaminase and deoxyadenosine deaminase activities in the *Cutaneous leishmaniasis* patients.

EXPERIMENTAL

Patients were selected from endemic area for *leishmaniasis* in Hatay. Diagnosis was confirmed by parasitology laboratory upon demonstration of the parasite in the lesions by direct smears. Lesions were cleaned with ethanol and punctured at the margins of the lesion with sterile lancet. Material was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain for examination by light microscopy. Patients with a negative smear were diagnosed by culture of the lesion (culture of fine needle aspirate on NNN (Novey-MacNeal-Nicolle) medium (with rabbit blood) and incubated at 24 ºC. Culture was observed for 4 weeks before being considered as negative[21-23]. Forty four patients in the age between 7-66 years were included for present study. They were divided into two groups. Untreated 19 active *Cutaneous leishmaniasis* patients (10 females, 9 males) and healed 25 *Cutaneous leishmaniasis* patients (13 females, 12 males) were treated using the drug with pentavalent antimonal compounds. Antimonate solution was administered intramusculary once a day for 15-20 d (20 mg/Sb/kg/d) by a single injection. 25 healthy subjects (13 females and 12 males) from the same area who were not exposed by *Leishmania* parasites were used as controls. All subjects were informed about the content of the study prior to tests and their written consents were obtained. The study protocol was approved by the ethical committee of the Mustafa Kemal University Hospital. After a 12 h night fast, 10 mL of venous blood samples from all patients and healthy controls were withdrawn and transferred into tubes without anticoagulants to measure the level of adenosine deaminase and deoxyadenosine deaminase activities. These blood samples were centrifugated for 15 min at 3500 rpm. Serum was removed and stored at -80 ºC for biochemical assays.

Serum adenosine deaminase and deoxyadenosine deaminase activities were estimated spectrophotometrically by the method of Giusti[24], which is based on the direct measurements of the formation of ammonia produced when adenosine deaminase and deoxyadenosine deaminase act in excess of adenosine and deoxyadenosine, respectively. Results were expressed as units per liter of serum (U/L). One adenosine deaminase enzyme unit was the amount of enzyme necessary to convert 1 μM of adenosine to inosine and ammonia and one deoxyadenosine deaminase enzyme unit was the amount of enzyme necessary to convert 1 μM of deoxyadenosine to deoxyinosine and ammonia per min at 37 ºC. Serum adenosine deaminase and deoxyadenosine deaminase activities were measured at 630 nm in a analyticjena specord-50 spectrophotometer with colourimetric assay in the laboratory of Department of Biochemistry, Pharmacy Faculty, Mersin University. The detection limit of the assay was 0-200 U/L with intra- and inter-assay coefficients of variables less than 4.5 % and 6 %, respectively. Adenosine deaminase activity in healthy subjects is usually within the 4-20 U/L range.

The data were analyzed using SPSS v16.0 (SPSS Inc, Chicago IL) software. For statistical analyzes, independent T test was carried out to compare adenosine deaminase and deoxyadenosine deaminase levels between control and non-treated patient groups, between non-treated patient and treated patient groups and lastly, between control and treated patient groups. Data were presented as mean ± standard deviation (± SD) and $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

The activities of adenosine deaminase and deoxyadenosine deaminase in the serum of control group, treated and non-treated patients are reported in Figs. 1 and 2. The mean level of adenosine deaminase and deoxyadenosine deaminase activities in serum of *Cutaneous leishmaniasis* patients at diagnosis was significantly higher ($p < 0.001$) than controls. Serum of treated patients showed that significant decreased mean adenosine deaminase and deoxyadenosine deaminase levels as compared with non-treated *Cutaneous leishmaniasis*

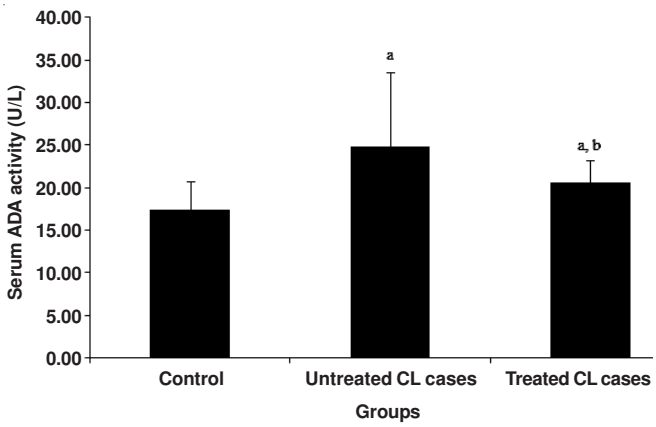

Fig. 1. Serum adenosine deaminase (ADA) activities in control, untreated and treated cutaneous leishmaniasis cases; [a]Significantly higher than control group ($p < 0.001$); [b]Significantly lower than untreated group ($p < 0.05$)

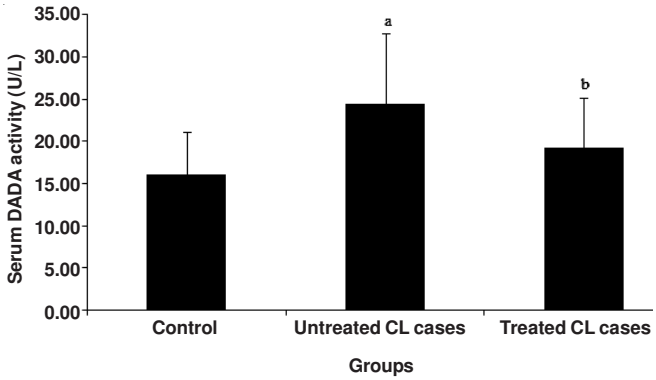

Fig. 2. Serum deoxyadenosine deminase (DADA) activities in control, untreated and treated cutaneous leishmaniasis cases; [a]Significantly higher than control group ($p < 0.001$); [b]Significantly lower than untreated group ($p < 0.05$)

patients ($p < 0.05$). Although, there was no significant difference between the control and treatment groups in deoxyadenosine deaminase activity ($p > 0.05$), adenosine deaminase activity was significantly higher in treated group than the control group ($p < 0.05$).

Adenosine deaminase is an enzyme that is required for lymphocyte proliferation, maturation and differentiation with detected biologic activity, particularly in T cells[2,17,18]. Adenosine deaminase activity is known to be increased in inflammatory diseases characterized by T-cell activation and proliferation and raised adenosine deaminase activity is found where cell-mediated immunity is stimulated[17,19,25-27]. Therefore, adenosine deaminase is considered a marker of T-cell activation; immune system dysregulation, T-cell and B-cell activation, immunoactive cell infiltration into the affected regions followed by accelerated neutrophil chemotaxis, phagocytosis and superoxide ($O_2.^-$) production, demonstrating the possible source of reactive oxygen species (ROS)[-28-30]. T-cell activation and neutrophil hyperfunction with excessive free radical injury were associated with increased lipid peroxidation in intracellular parasite infections, suh as plasmodium vivax and leishmaniasis[1,2,16,17]. Khambu *et al*.[31] and Baral *et al*.[32] described significantly increased adenosine deaminase activity in Nepalese visceral *leishmaniasis* patients as compare to healthy controls. The significantly high serum adenosine deaminase levels have been suggested in multibacillary leprosy and in patients of leprosy with reaction may be because of increased lymphoreticular activity during the reactional phases. The increase in adenosine deaminase activity in the patient group expresses phagocytic activity of macrophages and may have resulted from the erythrocyte damage caused by the parasite in the host[1,2,31,32]. Furthermore, Çulha *et al*.[33] found that lipid peroxidation and NO level increased in *Cutaneous leishmaniasis* cases. Since activated T lymphocytes cause neutrophil activation and neutrophils are one of the main ROS generation systems in these patients, our hypothesis is adenosine deaminase and deoxyadenosine deaminase enzyme activities could alter in *Cutaneous leishmaniasis* cases.

In our study, we found that adenosine deaminase and deoxyadenosine deaminase enzyme activities significantly increased in *Cutaneous leishmaniasis* cases. Also, Erel *et al*.[1] found increased lymphocytic specific adenosine deaminase activity in patients with *Cutaneous leishmaniasis*. Thus, increased production of adenosine deaminase and lipid peroxidaiton confirms the presence of an inter-relationship between T cells and neutrophils in leishmaniasis. Recirculation of activated T-cells and macrophages may cause higher serum adenosine deaminase activity in patients with *Cutaneous leishmaniasis* to avoid accumulation of toxic metabolites[2]. Barnes *et al*.[34] reported that adenosine could lessen the potentially damaging activity of neutrophiles at sites of infections. Therefore, the current hypothesis is that infiltration of T cells into the vascular wall followed by a neutrophil chemotaxis are associated with central changes in circulating neutrophils, with activation of these cells in *Cutaneous leishmaniasis*[1,2,26]. The increasing serum adenosine deaminase activity in patients with *Cutaneous leishmaniasis* may be a reflection of phagocytic activity of macrophages[2]. After the treatment with pentavalent antimonal compounds, adenosine deaminase and deoxyadenosine deaminase activities decreased compared to untreated group and their activities were near the control group levels. Probably, the immune system makes a magnificent effort to fight the parasites by increasing the adenosine deaminase levels unavailingly suggesting that there may be certain other essential factors needed to destroy the parasites and decrease antigenic stimulation[2,31]. On treatment there was a decrease in adenosine deaminase levels perhaps helping the immune cells to come to the normal stages. Presumably, resolution of the leishmanial infection occurred due to recovery of cell-mediated immune mechanisms following treatment[2,31].

Tripathi *et al*.[2] indicated high levels of adenosine deaminase activity in the sera of active visceral *leishmaniasis* patients as compared to controls suggesting that in spite of profound adenosine deaminase level in visceral *leishmaniasis* patients, still the disease progresses. Adenosine deaminase activities were found to be significantly decreased in follow-up cases after successful treatment. These findings are parallel to our results.

Measurement of adenosine deaminase and deoxyadenosine deaminase activities are used in the diagnosis and monitoring of autoimmune and inflammatory diseases due to its easy performance, high sensitivity, predictive, less cost can also be adapted to an autoanalyzer[14,18]. Therefore, since the increase in adenosine deaminase and deoxyadenosine deaminase activities reflect the enhanced functions of immunocompetent cells in response to inflammatory stimuli. It may be used as a simple biochemical test for rapid preliminary evaluation of the severity of disease and immune performance in *Cutaneous leishmaniasis*[1,14,18]. It may thus be useful in laboratories with limited resources, especially in new developing countries like Turkey where the incidence of *Cutaneous leishmaniasis* is very high and where, despite extensive work on it[1,18]. Evaluation of adenosine deaminase and deoxyadenosine deaminase activities in the serum of patients with *Cutaneous leishmaniasis* can be considered a useful tool for monitoring their clinical status[14,18]. Adenosine deaminase activity in serum of patients with *Cutaneous leishmaniasis* suggested that monitoring Adenosine deaminase levels in *Cutaneous leishmaniasis* might provide a useful guide for the interpretation of infection status. Therefore, Adenosine deaminase may be a predictive and sensitive parameter of leishmaniasis treatment.

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