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Short Communication

Role of Adenosine Deaminase in Patients with Erythematotelangiectatic Rosacea and *Demodex folliculorum* Positivity

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Abstract

Background: Adenosine deaminase (ADA) is an aminohydrolase involved in the catabolism of purine nucleotides and irreversibly deaminizes adenosine and deoxyadenosine to inosine and deoxyinosine. ADA enzyme deficiency results in the loss of functional properties of B and T lymphocytes. *Demodex* species have been reported to be transmitted between humans through close contact and to play a role in the pathogenesis of rosacea, acne vulgaris, perioral dermatitis, seborrhoeic dermatitis, micro-papillary-pruritic dermatitis and blepharitis. The present study aimed to compare serum ADA levels in *D. folliculorum* positive patients with the healthy control individuals.

Methods: Serum ADA levels were examined for 30 patients diagnosed with erythematotelangiectatic rosacea and 40 healthy individuals in Malatya Inonu University in 2017. Standardized skin surface biopsy (SSSB) method was used to diagnose *D. folliculorum*. A significant decrease was found in the ADA levels of *Demodex*-positive rosacea patients when compared to the control group.

Results: ADA levels were decreased in the *Demodex*-positive group. The mean ADA level in patient group was significantly lower than the mean in the control group ($P < 0.001$). There was no significant difference between the patient and control groups in terms of age and gender.

Conclusion: During and after treatment of *Demodex*-positive rosacea patients, determination of ADA levels may give more detailed information on the immune mechanisms.



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Introduction

D. *folliculorum* and *D. brevis*, which live at the base of hairs and in the fat glands of the skin especially in facial follicles, are the most common permanent ectoparasites encountered in humans. *Demodex* spp. are found in different areas of the body where hair grows; mainly in the nasolabial region, base of the eyebrows, chin and forehead and less often in the outer ear, nipples, back, penis and hip (1-4). For diagnosis, methods such as cellophane tape, skin scratch, punch biopsy and standard superficial skin biopsy (SSSB) are used (5,6).

With dermatoses such as rosacea, perioral dermatitis, Grover's disease and eosinophilic folliculitis, there is an increase in parasite numbers observed. Transmitted by close contact between people, it has been reported by researchers that they play an important role in the pathogenesis of rosacea, acne vulgaris, perioral dermatitis, seborrheic dermatitis, macropapular-itchy dermatitis and blepharitis (1,3,4). Some researchers think the presence of the parasite in pilosebaceous follicles is harmless while others argue that the parasite has a part in the etiopathogenesis of some cutaneous diseases that develop on the face (7-9).

Dysfunction of sebaceous glands, T cell suppression and external factors have been blamed in *Demodex* infections. HLA Cw2 and Cw4 haplotypes were found to be involved in the development of the clinical symptoms of the disease (10). In Cw2 and Cw4 expression, NK2 cells are attracted to the centre of inflammation, causing suppression of Th1 response and inhibition of the lysis of the parasite. Besides, it contributes to the survival of the parasite by damaging cutaneous cells (11). Similarly, it was reported in histological examinations that the parasite could cause mononuclear and perifollicular inflammatory infiltration. The resulting infiltration was observed to arise from CD4+ T lymphocytes and CD8+ T cells. Furthermore, CD1a+ macro-

phages were detected around the infested follicle (9). Volmer also described folliculitis in 83% of follicles harboring *Demodex* (12).

Adenosine deaminase is an aminohydrolase which has a role in the catabolism of purine nucleotides. Adenosine deaminase (ADA) is a basic enzyme for the monocyte-macrophage system and the proliferation and differentiation of lymphocytes. The structural gene for adenosine deaminase is on the 20th chromosome, it is located in the form of multiple molecular in human tissue, and it has a broad distribution in the form of multiple cells. In mammals, it catalyzes the adenosine, deoxyadenosine and the known ribosides. ADA activity is 10 times more common in lymphocytic cells, in comparison to erythrocytes (13, 14).

Rosacea is a common facial dermatosis characterized by recurrent blushing attacks, erythema, telangiectasia, papules and pustules. In the most recent classification, four subtypes of rosacea have been defined as erythematotelangiectatic (vascular), papulopustular (inflammatory), phymatous (hyperproliferative) and ocular (ophthalmologic) rosacea (15).

The more common initial symptom of rosacea is episodic erythema affecting the central portion of the face (central facial flushing). There is generally, no itching, however burning or stinging sensations can reach serious levels. The main triggers are sun, cold weather, hot drinks, spices, alcohol and sudden emotions. With continuously lengthening duration, flushing attacks eventually develop into permanent erythema. In addition to erythema, telangiectasia, papules and pustules are commonly observed symptoms. In a small proportion of patients, connective tissue hypertrophy, sebaceous gland hyperplasia and chronic lymphedema phymatous rosacea. Additionally, the incidence of eye involvement in rosacea is very high. The etiology of rosacea has not been fully explained, but hormonal factors, gastrointestinal system disorders, nutritional

factors, medications, sun damage, emotional factors, *D. folliculorum* infestation, *Helicobacter pylori* infection and immune system dysfunction are thought to play a role (15).

Cellular response is known to be activated in *Demodex* infections. We aimed to compare serum ADA levels in *D. folliculorum* positive patients with the healthy control individuals.

Materials and Methods

The current research design was an observational cross sectional study conducted in Malaty Inonu University in 2017. The study was approved by the Ethics Committee of Inonu University Medical Faculty (Date: 02.10.2007, the protocol number: 2007/146), and only the patients who volunteered to provide samples were evaluated.

SSSB method was used to diagnose *D. folliculorum*. SSSB is a non-invasive method of investigation, applied by taking the superficial layer of the skin and follicular contents with the aid of cyanoacrylate adhesive (6,14). Observing more than 5 /cm² *Demodex* on SSSB is significant for definite diagnosis (5,6). For application, a drop of cyanoacrylate adhesive is placed on a clean slide. The adhesive is touched to the facial region for sampling (forehead, cheek and nasal dorsum) with slight pressure and left for 1 min. Then the slide is removed in a single motion and 2.3 drops of entellen immersion oil or glycerine are dropped onto the sample. The sample is covered with a cover slip and examined with a light microscope using 4x and 10x objectives. In the study, if the number of the living parasites in one cm² area is five or more, it has been accepted as the proof of this demodiosis (8,16).

Considering that serum ADA levels may change in parasitic diseases, the intestinal parasites in the patient and control groups were studied using native lugol, perianal area material taken by cellophane tape and sedimentation methods. Thirty patients who were *Demodex*-

positive formed the patient group having erythematotelangiectatic rosacea (ETR). Of the *Demodex*-positive patients, those who had other parasites in the faeces, who were on hormone medication, as well as those who were smoking and using alcohol were excluded from the study, as these factors may change ADA levels. Of the people who volunteered to take part in the study, those who did not have any parasitic infection, did not smoke, were not on any hormone medication and did not use alcohol were included in the study as the control group. After the patients who were found to have parasites by SSSB were duly informed, 5 ml of blood samples were taken, sera of the samples were separated and stored at -20 °C. Additionally, forty healthy individuals included in the control group were found to be *Demodex*-negative by SSSB.

Ellis and Goldberg methods were used for measuring of the ADA levels of the samples (13). The ammonium ion, which is released from adenosine by the action of adenosine deaminase creates the green blue colored indophenol complex as a result of Berthelot reaction. The intensity of the resulting color is increased in proportion to the enzyme concentration in the medium. This complex was seen at 632 nm wavelength in the spectrophotometer.

Statistical Analysis

According to the power analysis, minimum group sample sizes of 30 and 30 (in each group) achieved 95.0% power to reject the null hypothesis of equal means when the mean difference was 10.09 with standard deviations of 4.95 for patient group and 14.07 for control group, and with a significance level (alpha) of 0.05 using a two-sided two-sample unequal-variance *t*-test. The data were explained as mean \pm standard deviation or frequencies where it was appropriate.

The normality test was performed with the Shapiro-Wilk test. Independent sample *t*-test was used for the statistical analysis. The $P < 0.05$ values were considered statistically signif-

icant. In the statistical analysis, the SPSS (Chicago, IL, USA) 22.0 package program is used.

Results

The patients, of whom 14 were male and 16 were female, had a mean age of 38.6 ± 7.07 . In the control group composed of 40 *Demodex*-negative healthy individuals, 18 were male and 22 were female and had a mean age of 40.23 ± 7.01 yr. There was no significant difference between the patient and control groups in terms of age and gender.

A significant decrease was found in the ADA levels of the *Demodex*-positive patient group when compared to the control group ($P < 0.001$). The mean ADA level was found as 11.02 ± 4.95 U/L in the patient group and 21.11 ± 14.07 U/L in the control group. The mean ADA level in patient group was significantly lower than the mean in the control group.

Discussion

Recent reports of demodicidosis related with AIDS and malignancies suggested that host immune dysfunction might allow proliferation of the normally commensal mites with subsequent disease production (17).

Reduced or absent ADA activity results in failure of DNA synthesis and inhibition of precursor T-cell maturation related with a seriously combined immunodeficiency syndrome; these findings demonstrate the significance of ADA activity for T-cell maturation (18).

In case of increased serum ADA deficiency, both cellular and humoral immunity is in an impaired condition. ADA activity was established to be critical in normal lymphocyte function. Adenosine deaminase enzyme, which is accepted as a T cell marker, is elevated in body fluids and plasma in case of diseases where cell-mediated immune stimulation occurs (18,19). ADA activity is increased in

autoimmune diseases such as typhoid, acute pneumonia, brucellosis, infectious mononucleosis, tuberculosis, sarcoidosis, liver diseases, acute leukemia, various malignancies and rheumatoid arthritis, systemic lupus erythematosus (SLE) and Behçet's disease in which the cellular immunity was stimulated (20,21). Besides, it was argued that ADA levels increased in patients with primary immune deficiency (Leukocyte adhesion deficiency, hyper IgM and Wiskott-Aldrich Syndrome, chronic granulomatous disease) (22).

In this study, a significant decrease was established in the ADA levels in *Demodex*-positive erythematotelangiectatic rosacea patients. Similarly, ADA activity was found to fall in visceral leishmaniasis (23). Similarly, Karaman et al detected a significant drop in the ADA activity of patients with seropositive *Toxoplasma gondii* and *Giardia intestinalis*, according to healthy controls. The decrease in ADA level may be attributed either to the fact that by virtue of being dated, toxoplasmosis infection failed to elevate T lymphocytes or to increased oxidative stress in parasitic infections (24). ADA activity increases in cases of immune system activation and decreases in cases of immune system suppression. In the present study, *Demodex*-positive patients had been on erythematotelangiectatic rosacea treatment for a long time. Therefore, since T lymphocytes involved in the cellular immune response did not increase, a decrease in ADA levels might have observed. It was reported that human demodicosis might mimic many other inflammatory dermatoses, such as folliculitis, rosacea and perioral dermatitis and was associated mainly with immunosuppression (25). Based on these results, during and after treatment of *Demodex*-positive rosacea patients, determination of ADA levels may give more detailed information on the immune mechanisms.

Rosacea is a common chronic dermatosis defined by varying degrees of redness, erythema, telangiectasia, edema, papular, pustular and ocular lesions and skin tumors. The dis-

ease may be caused by genetic susceptibility, abnormal vascular reactivity, variations in vascular mediatory mechanisms, *D. folliculorum* infestation and other factors (26). One study took standard superficial skin biopsies from 80 patients with rosacea and investigated in terms of *Demodex*. The study identified that *Demodex* mites may play a role in the inflammatory reaction of acne rosacea (27). Another study investigated the correlation between the sebum level and the density of *D. folliculorum* in patients with erythematotelangiectatic rosacea and stated that *D. folliculorum* may play a role in the etiology of rosacea (28).

Mean ADA levels in *Demodex* positive rosacea patients was clearly low compared to the mean of the control group. In the literature search, we could not found a study investigating ADA levels in *Demodex* spp. According to the study data, this situation shows that measurement of ADA levels may be a biochemical parameter that aids in clinical diagnosis of rosacea patients with suspected *Demodex*. Thus, ADA levels may be monitored by clinical dermatologists and aid in the diagnosis and treatment of rosacea patients with suspected *Demodex*.

Conclusion

More detailed-information might be obtained about mechanisms of immune system in further controlled clinical trials by determining ADA levels in both *Demodex*-positive and *Demodex*-negative rosacea patients. In addition, during and after treatment of *Demodex*-positive rosacea patients, determination of ADA levels may give more detailed information on the immune mechanisms.

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Conflict of interest

The authors declare that there is no conflict of interest.

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