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## Serum adenosine deaminase activity in cutaneous anthrax

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**Background:** Adenosine deaminase (ADA) activity has been discovered in several inflammatory conditions; however, there are no data associated with cutaneous anthrax. The aim of this study was to investigate serum ADA activity in patients with cutaneous anthrax.





**Material/Methods:** Sixteen patients with cutaneous anthrax and 17 healthy controls were enrolled. We measured ADA activity; peripheral blood leukocyte, lymphocyte, neutrophil, and monocyte counts; erythrocyte sedimentation rate; and C reactive protein levels.

**Results:** Serum ADA activity was significantly higher in patients with cutaneous anthrax than in the controls ( $p < 0.001$ ). A positive correlation was observed between ADA activity and lymphocyte counts ( $r = 0.589$ ,  $p = 0.021$ ) in the patient group.

**Conclusions:** This study suggests that serum ADA could be used as a biochemical marker in cutaneous anthrax.

**MeSH Keywords:** **Adenosine Deaminase – diagnostic use • Anthrax – diagnosis • Diagnosis**

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## Background

Anthrax is a rare, potentially fatal zoonotic disease caused by the bacterium *Bacillus anthracis*, which can infect both animals and humans [1]. Infection via inhalation of *Bacillus anthracis* spores can result in a mortality rate of up to 96% [2]. Major routes of exposure and subsequent infection have been confirmed through skin contact with, or the inhalation or ingestion of, *Bacillus anthracis* spores [3]. Although West Africa has the highest incidence in the world [4], anthrax is also a significant problem in Central America, Romania, Greece, and Turkey [4–6].

Cutaneous anthrax, the most common form of the disease, accounts for 95% of anthrax cases [7]. It is acquired when spores enter through a cut or abrasion in the skin. The most common areas of exposure are the hands, arms, face, and neck [8]. Diagnosis may have to be on a clinical basis in the majority of patients. Unfortunately, the diagnosis of cutaneous anthrax can be difficult, particularly in endemic regions, because of its variable clinical phenotype.

Adenosine deaminase (ADA), an enzyme present in red blood cells and blood vessel walls, catalyzes the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine. Inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and, finally, to uric acid [9]. This enzyme is considered a suitable biochemical marker of cell-mediated immunity [10]. ADA has been accepted as an important enzyme in the proliferation and differentiation of T-lymphocytes, as well as for the maturation and function of blood monocytes and macrophages [11]. Its levels are 10 times higher in T-lymphocytes than in erythrocytes, and it is frequently considered a noninvasive diagnostic test for tuberculosis, with 90–100% sensitivity and 89–100% specificity [12].

The aim of this study was to investigate serum ADA activity and other inflammatory markers in patients diagnosed with cutaneous anthrax in the Van region of Turkey, where the disease is prevalent.

## Material and Methods

All patients (5 males and 11 females) were diagnosed with cutaneous anthrax at outpatient clinics of the Department of Infectious Diseases and Clinical Microbiology (Medical Faculty, Yuzuncu Yil University, Van, Turkey) in a 2-month period (1 November–31 December 2013) and 17 healthy controls (11 males and 6 females) were without a history of chronic or recurrent disease and had normal physical examination results.

All of the cutaneous anthrax cases were characterized by evolving skin lesions and had a history of animal contact. The

diagnosis of cutaneous anthrax was based on dermatologic findings, including papules from the vesicular stage; pruritic ulcers covered by characteristic black eschar; and edema, erythema, or necrosis without ulceration. Cases were confirmed by a positive smear or by isolation of *Bacillus anthracis* in clinical specimens [13]. *Bacillus anthracis* isolates were identified on the basis of conventional methods, such as gram-positive bacilli with spores present in the smear, the presence of a capsule, the lack of motility, or catalase positivity.

The study protocol was approved by the local ethics committee. All subjects were informed about the study and written consent was obtained from each subject.

### Exclusion criteria

The exclusion criteria included a history of alcohol abuse, habitual smoking, intravenous drug abuse, pregnancy, antioxidant supplement use, hypertension, diabetes mellitus, liver or renal disease, rheumatoid arthritis, pulmonary disease, and coronary artery disease.

### Blood samples

Blood samples were collected in tubes at 8:00 a.m. and 11:00 a.m. after an overnight fasting period and were immediately stored at 4°C. Peripheral blood leukocyte, lymphocyte, neutrophil and monocyte counts, and erythrocyte sedimentation rates (ESR) were measured. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min, and C reactive protein (CRP) levels were measured. The serum samples were stored in plastic tubes at –80°C and used to analyze ADA activity.

### Measurement of serum adenosine deaminase activity

Serum ADA activity was measured with an enzymatic spectrophotometric method determined by Giusti and Galanti, which is based on the direct measurement of ammonia formation, a byproduct that forms when ADA is in the presence of excess adenosine [14]. The results are expressed as units per liter (U/L).

### Other parameters

The serum CRP levels were determined by using commercially available assay kits (Roche®, Mannheim, Germany) with an autoanalyzer (Roche®/Hitachi modular P-800, Mannheim, Germany).

Complete blood counts were performed using a Celdyne 3700 Hematology Analyser (Abbott®).

ESR was measured using an automated chemiluminescence autoanalyzer (Roche®).

**Table 1.** Laboratory findings and demographic characteristics of the two groups\*.

Parameters	Patients (n=16)	Controls (n=17)	p
Age (years)	44±6	41±3	0.211
Sex (female/male)	5/11	6/11	0.805
Leukocyte counts (mm <sup>3</sup> /ml)	8.8±2.3	7.2±1.8	0.037
Lymphocyte counts (mm <sup>3</sup> /ml)	1.9±0.8	1.5±0.5	0.099
Neutrophil counts (mm <sup>3</sup> /ml)	6.1±2.5	4.7±1.6	0.070
Monocyte counts (mm <sup>3</sup> /ml)	0.6±0.2	0.5±0.2	0.987
ESR (mm/h)	31.9±15.5	12.5±4.6	0.001
CRP (mg/dl)	29.7±17.5	3.2±1.5	0.001
ADA (U/L)	31.9±1.6	17.5±1.4	0.001

\* Values are mean ± standard deviation. ESR – erythrocyte sedimentation rate; CRP – C reactive protein; ADA – adenosine deaminase.

### Statistical analysis

The results are expressed as the mean ± standard deviation. Parameter comparisons of patients and healthy controls were performed using Student's t-test. Correlation analyses were performed using Pearson's correlation test. The results were considered to be statistically significant when the p-value was less than 0.05.

### Results

The demographic characteristics of the patients with cutaneous anthrax and the controls are shown in Table 1. There were no significant differences between the groups with respect to age and sex ( $p>0.05$ ) (Table 1).

Blood leukocyte counts were significantly higher in the patients with cutaneous anthrax compared with the controls ( $p=0.037$ ). Although lymphocyte and neutrophil counts were higher in the patient group, there were no statistically significant differences ( $p>0.05$ ). ESR and CRP levels were significantly higher in the patients with cutaneous anthrax compared with the controls ( $p<0.001$ ) (Table 1).

Serum ADA activity was significantly higher in patients with cutaneous anthrax than in the control subjects ( $p<0.001$ ) (Table 1).

A positive correlation was observed between ADA activity and lymphocyte counts ( $r=0.589$ ,  $p=0.021$ ); however, there was no correlation between ADA and the other inflammatory markers investigated ( $p>0.05$ ).

### Discussion

We evaluated the serum ADA activity in patients with cutaneous anthrax. In the diagnosis of cutaneous anthrax, the clinical

presentation of the disease and a history of close contact with sick animals are very important. If a patient has a typical malignant pustule or malignant edema and has had a history of contact with animals, the diagnosis may be easy. The clinical laboratory diagnosis of cutaneous anthrax is generally established by conventional microbiological methods, such as bacterial cultures and directly gram staining smears of clinical specimens [15]. However, the clinical presentation could be atypical and the patient's recollection of contact with animals may not be accurate, or the patient may neglect to supply this information. In these situations, the diagnosis of cutaneous anthrax might be difficult. PCR, immunohistochemistry, laboratory parameters (e.g., CRP, ESR, and white blood cell [WBC]), and anthracin skin tests may be helpful in the diagnosis of anthrax [16,17].

Inflammatory markers such as ESR and WBC count were found to be high in some patients [18]. However, the elevation of CRP levels in patients with cutaneous anthrax, as we found, has not been reported previously.

ADA is a key enzyme in purine metabolism; it converts adenosine and deoxyadenosine to inosine and deoxyinosine by irreversible deamination [19]. ADA is widely distributed in human tissues (its highest activity being in lymphoid tissues), and it is primarily associated with T-lymphocyte proliferation [20]. Although ADA has been considered a nonspecific marker of T-cell activation, the precise mechanisms by which serum ADA activity is altered have not been clearly identified [21].

High levels of serum ADA have been reported in infectious diseases such as viral and bacterial pneumonia, HIV infection, extra-pulmonary and pulmonary tuberculosis, *H. pylori*, acute appendicitis, visceral leishmaniasis, and mononucleosis [11,12,22,23] and might have diagnostic value. Moreover, circulating levels of ADA have been shown to increase in several inflammatory conditions, including Behçet's disease, systemic

lupus erythematosus, rheumatoid arthritis, and certain malignancies, especially those of hematopoietic origin [24–34].

## Conclusions

Although to our knowledge this is the first study to investigate serum ADA enzyme activity in patients with cutaneous anthrax, it has a major limitation. Our study design did not allow us to investigate whether ADA activity is uniquely associated with cutaneous anthrax. Ideally, a third group of sick patients, with elevated CRP or ESR, but without lymphocytes or ADA levels,

could have been included to highlight the issue. However, in the current study, we observed that serum ADA enzyme activity was significantly higher in patients with cutaneous anthrax than in the healthy controls. Moreover, we found that acute cutaneous anthrax patients had increased WBC, CRP levels, and ESR compared with the control group. These results suggest that ADA contributes to the inflammation seen in cutaneous anthrax and might be used in the clinical setting.

## Conflicts of interest

The authors report no conflict of interest regarding this work.

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