# Adenosine Deaminase values of serum and erythrocytes in patients with Cutaneous Leishmaniasis and healthy subjects in Nineveh state population

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**Abstract**

Cross sectional, hospital based study was carried out on 46 Cutaneous Leishmaniasis patients (25 males and 21 females) and fifty four healthy volunteers were investigated to serve as normal subjects. Serum and erythrocytes Adenosine Deaminase activity was assessed spectrophotometerically by using colorimetric method. Serum and erythrocytes Adenosine Deaminase activity of Leishmaniasis-patienats were highly significant ($p< 0.005$) and higher (38.8 ± 2.5 and 188.1 ± 23.3 U/L) than that values of healthy individuals (14.8 ± 1.4 and 64.1 ± 7.5 U/L respectively). No significant difference values were found in blood ADA activity according age and sex. In conclusion serum and erythrocytes ADA values may be used in the follow up of patients with Cutaneous Leishmaniasis together with clinical and other laboratory findings.

# قيم مصل وكريات الدم الحمر لانزيم الادينوسين دي امينيز لمرضى اللشمانيا والأصحاء في محافظة نينوى

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**المستخلص**

ستة واربعون عينة دم جمعت من مرضى اللشمانيا الجلدية وكانت العينات موزعة بواقع 25 عينة ذكرية و21 عينة انثوية , كما جمعت 54 عينة دم طبيعية أستخدمت عينات سيطرة لهذه الدراسة, جمعت من متطوعين أصحاء. تم تحديد فعالية انزيم الادينوسين دي امينيز في مصل ومتحلل كريات الدم الحمراء باستخدام الطريقة اللونية. اذ وجدت فروقات معنوية معنوية (عند مستوى احتمالية اقل من 5%) بين نشاط انزيم الادينوسين دي امينيز بين مرضى اللشمانيا والاصحاء فكانت فعالية الادينوسين دي امينيز المقاسة بالطريقة اللونية من مصل ومتحلل كريات الدم للاصحاء هي (14.8 ± 1.4) و(64.1 ± 7.5 ) وحدة لكل لتر على التوالي اما فعالية الانزيم في مصل ومتحلل كريات الدم في المرضى فهي (38.8 ± 2.5 ) و(188.2 ± 23.3) وحدة لكل لتر على التوالي, تبين انه هناك فروقات معنوية (عند مستوى احتمالية اقل من 5%) بين مستوى فعالية الانزيم لمصل ومتحلل كريات الدم المرضى والاصحاء. و لم فروقات معنوية في ألفعالية نسبة الى العمر والجنس.

## Introduction

Adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine to inosine and ammonia (1,2)ADA is considered to be one of the enzymes involve in the catabolism of the purine bases of the purine salvage pathway and also considered as a key enzymes in purine nucleotide degradation to yield uric acid as the catabolism end product (3,4). It has been suggested that detoxification of adenosine and deoxyadenosine might Such as brucellosis and rheumatoid arthritis (6,7,8).In order to know diagnostic phenomena, most of the enzyme tests required for diagnostic purpose determine enzyme activities in plasma or serum, although similar principles applied for all biological material (tissue,urine, cerebrospinal fluid ,liquor , cellculture,etc).

In general,plasma enzymes activities represent a steady state in wihich the rate of release from cells into plasma and the rate of removal from them are equal to explain these phenomena, there are several theoretical mechanism, by which serum enzyme levels might be increased in tissue that have normal content, and may be released This is used in the diagnosis of different pathogens cases, also help to detect and localize tissue cell damage to monitor treatment and progress of various diseases (8, 9). Leishmaniasis is adisease caused by a parasite and spread by the bite of infected sand flies. There are several different forms of leishmaniasis. The most common is the skin form (Cutaneous Leishmanisis) which cause scarring skin sores. Leishmanisis exists in Iraq, Kuwait, Afghanistan, and other places in the Middle East. Sand fly life season in Iraq runs from April through November and peaks in September or October (10).The aim of this study was to evaluate the activity of Adenosine Deaminase in lieshmaniasis patients blood in relation to healthy individuals in Nineveha state population which is used as a parameter for the diagnosis of different clinical cases.

## Material and Methods

Forty-six patient subjects distributed as 25 males and 21 females and fifty four healthy volunteers.Blood samples were collected from Mosul , Talafer, and Al Ayadyaa hospitals , which are hyperendemic area for leishmaniasis in the northern of Iraq. The clinical diagnosis was confirmed by laboratory, patients presenting with cutaneous lesions only were clinically assessed and each patient underwent a through physical examination. Biopsies taken from lesions were used to prepare impression smears (stained with buffered Giemsa) after diagnostic procedure; blood sample was withdrawn to measure serum and erythrocyte ADA. Blood sample collected from venous blood about (5-7) ml was drawn from the cubital vein using disposable needles and syringes, without using tourniquet. The blood sample was divided into two part: one part for estimation serum ADA, by put amount of blood in a clean dry plain plastic tube and was allowed to clot for 10-25 min before centrifugation, and then centrifuge at 3000 rpm for 15 min, the clear serum were transferred to clean plastic tubes and topped by plastic stoppers and stored at-20 $^{0}$C till time of analysis, the second part of blood sample used for estimation of erythrocyte ADA, by using tube contain sodium citrate as a anticoagulant after

centrifugationplasma ,buffy coat and the upper most layer of erythrocytes were removed, the remaining An aliquot of washed erythrocyte was used to analyze RBCs ADA.

ADA was measured by using Calanti and Gusti method (11) .Which is calorimetric methods, they based on the indirect measurement of the formation of ammonia produced when adenosine deaminase acts in an excess of adenosine to produce inosine. The release of ammonia was determined hypochlorite and phenol in alkaline solution. While the using of sodium nitroprusside is a catalyst. The ammonia concentration is directly proportional to the absorbance of the indophenols. Light absorbance of produced indophenols was measured as 628 nm. And the enzyme activity was expressed as in international Unit/Liter. (I.U/L or U/L), Statistical analysis Student's *t* tests were performed in the statistical package computer program for social sciences (Spsscomputer program).all result is expressed in the form of mean values ± standard deviation (SD), erythrocytes were washed three times with about 10 volumes of ice-cold $MgCl_2$ solution (112 mmol/L).

with statistical significance differences at probability less than 5%, ($P< 0.05$) respectively

## Results

Serum and erythrocytes adenosine deaminase activity of patients and healthy individuals are shown in Table (1). The mean serum ADA in cutaneous leishmaniasis (38.8 ± 2.5 U/L) was significantly ($p<0.005$) higher than that value of individuals (14.8 ± 1.4 U/L). also, the value of erythrocyte ADA in patients (188.9 ± 23.3 U/L) was significantly higher than that value of normal subjects (64.1 ± 7.5 U/L); No significant differences were found in serum and erythrocyte ADA between males and females normal subjects (Figs. 1 and 2) also no significant differences were found according age group of normal subjects (figs. 3 and 4).

**Table (1): Serum and erythrocyte adenosine deaminase activity in patients with cutaneous leishanisasis and healthy subjects.**

| | Leishmaniasis- patient group | | | Healthy control group | | |
|---|---|---|---|---|---|---|
| | Range | Mean | SD | Range | Mean | SD |
| sADA | 33.4 – 43.4 | 38.8 | 2.5* | 12.1-18.4 | 14.8 | 1.4* |
| rADA | 106.6 – 216.3 | 188.2 | 23.3* | 49.3- 76.1 | 64.1 | 7.5* |

**Figure (1): Sex relationship with the serum ADA activity in normal subjects**

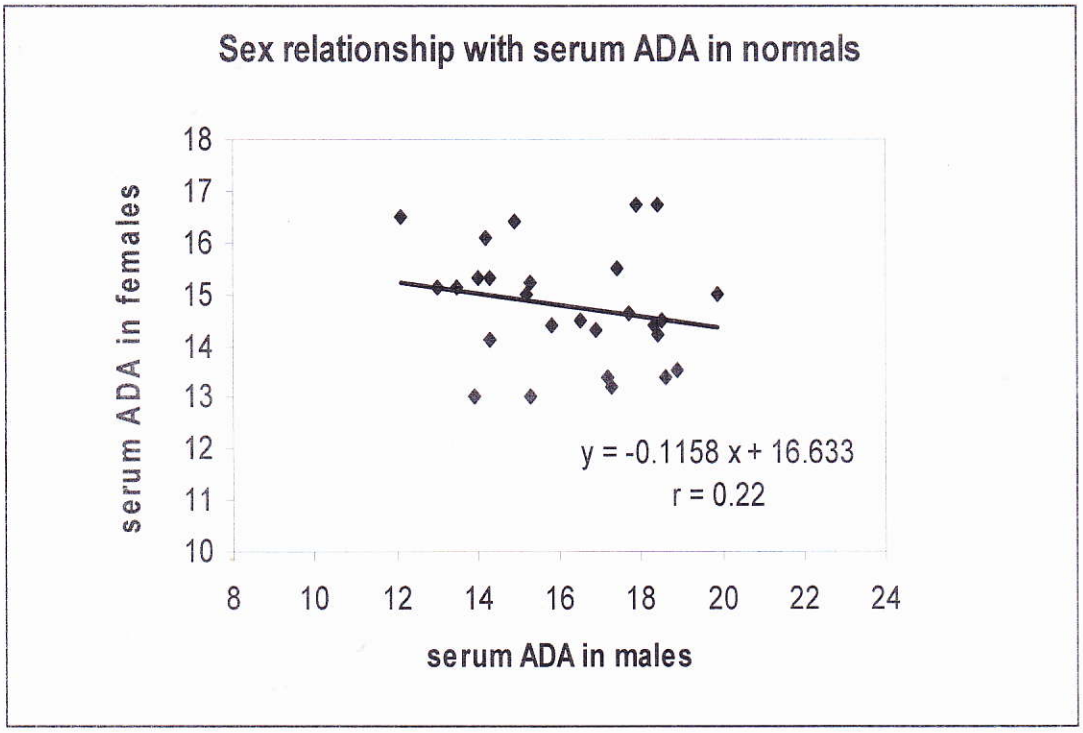



**Figure (2): Sex relationship with the erythrocyte ADA activity**

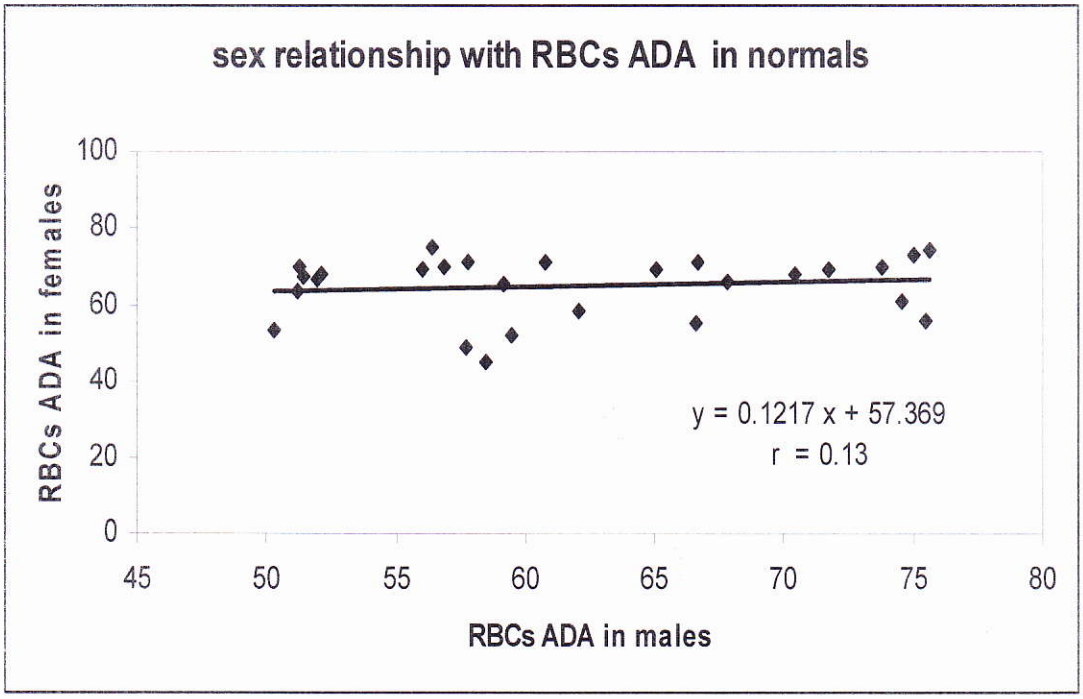


**Figure (3): Age relationship with the serum ADA activity**

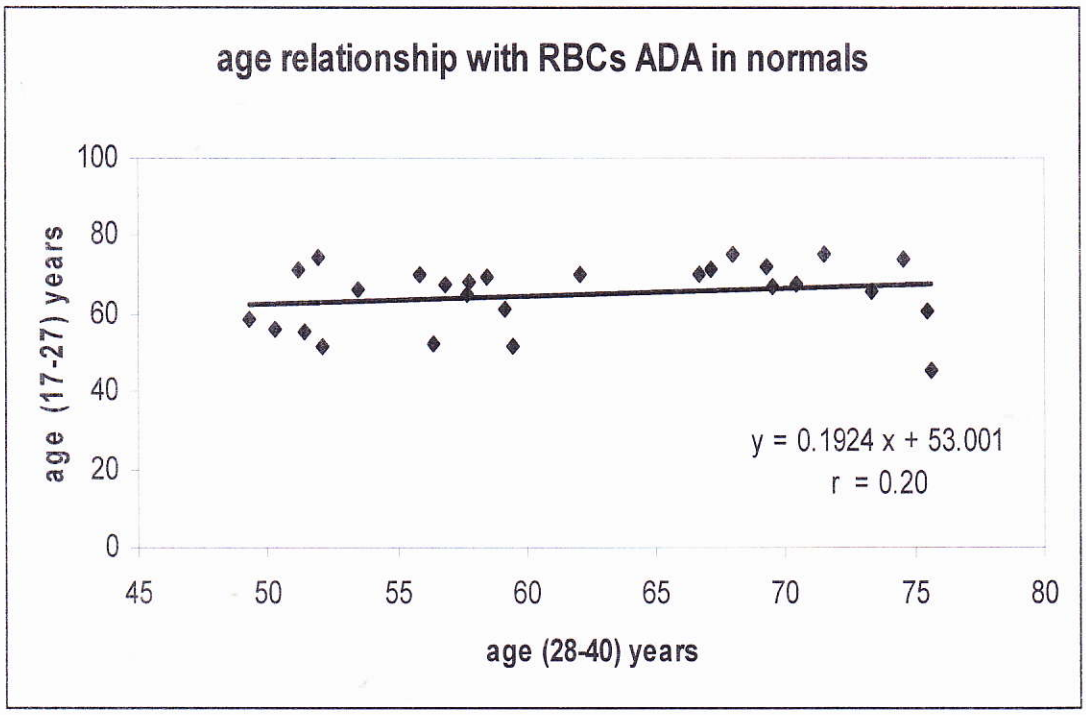
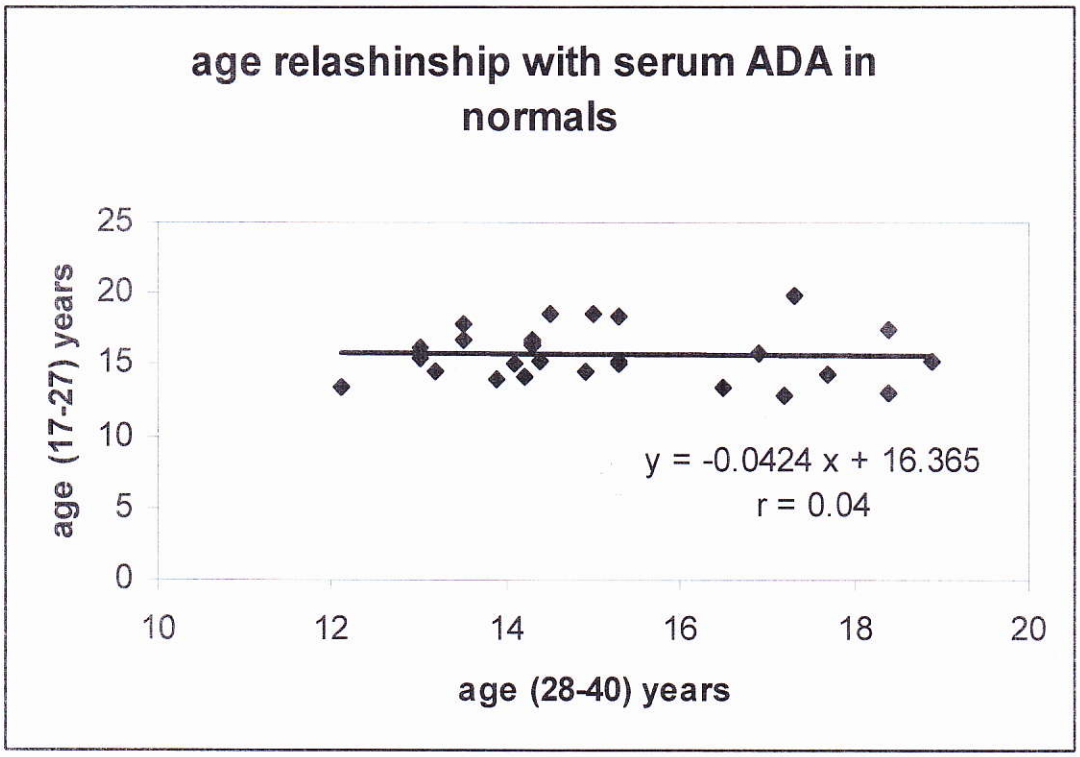



**Figure (4): Age relationship with the erythrocyte ADA activity in normal subjects**



## Discussion

It is generally agreed that adenosine deaminase is an enzyme catalyzes the irreversible hydrolytic deamination of adenosine and deoxyadenosine and converted them to toxic derivatives, involved the purine metabolism, in combination with the nucleotide phosphorylase and xanthine oxidase reactions, the adenine part of adenosine can be converted to toxic uric acid (6, 12). ADA is present in blood (serum and erythrocyte) and most ody tissues, particularly lymphoid tissues. And is essential for the maturation and function of lymphocytes, especially those of T lineage and is required for the maturation of human blood monocytes to macrophages (13) . Elevated Adenosine deaminase value can be used as a marker for many dieeases that associated with high lymphocyte .High ADA was shown in serum and red blood cells of various diseases including infection , malignancy and liver disease (14). Increased serum adenosine deaminase activity has been observed in patients with visceral leishmaniasis (Kala azar)(15) .In current study , serum adensine deaminase levels were significantly ($p<0.005$) higher in the cutaneous leishmaniasis (38.84±2.53 U/L) than those of healthy individuals (14.76± 1.43 U/L),while Erythrocyte ADA values were significantly higher in patients (188.16 ± 23.33 U/L) than that value of normal subjects (64.07 ± 7.55 U/L). *Leishmania* obligate intracellular parasites of macrophages, it is generally assumed that the main effector mechanism controlling parasite growth is lymphokine-mediated macrophage activation so, increasing serum adenosine deaminase activity in patients with cutaneous leishmaniasis may be reflect of phagocytic activity of macrophages(16). On the other hand, Red blood cell ADA activities increased on the order of a hundred-fold affected individuals. The basic abnormality appears to result from overproduction of structurally normal enzyme due to abnormal translation efficiency. Also increased ADA in erythrocytes may be due to an increase in steady-state levels of ADA mRNA of normal sequence .(17) . In this study, we found that there is no effect of age and sex on ADA activity in normal subjects and increased of adenosine deaminase levels in leishmaniasis depends upon the severity of the disease.

More recently ADA is regarded one of the best sign and considered to be a good parameter, which is used in the diagnosis of different pathogens cases, also help to detect and localize tissue cell damage to monitor treatment and progress of disease. And to explain the cause and mechanisms of increased levels of ADA activities in serum and erythrocytes cutaneous leishmaniasis further studies are requested, all these information's assist the enzyme to be used both effectively and economically as a parameter for the diagnosis of different clinical cases .

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