# The Kinetic Study of Adenosine Deaminase Activity in Renal Failure Patients

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## Abstract

Serum adenosine deaminase levels was measured in patients with acute renal failure and compare them with the results of healthy individuals. Serum adenosine deaminase levels were found to be significantly higher in patient with acute renal failure than in healthy individuals (63±20.1U/L) and (28±9.2IU/L), respectively. The study was carried in optimum pH value (6.5) and optimum temperature 37ć by which enzyme possess highest activity, the activation energy (Ea) of the reaction (ES-Complex) formation was estimated.

## Introduction

Acute renal failure occurs in 5 percent of hospitalized patients in certain situations, such as when a patient has glomerular diseases, macro vascular diseases or obstructive diseases, rapid cause's renal damage and nephrotoxines. Death is most often caused by infection or cardiorspiratory (Nolan & Andrson, 1998; Brady & Singer, 1995). Arise in serum adenosine deaminase activity (SADA) is usually taken to indicate either hepatic diseases or hepatic microsomal enzyme induction (Ocana *et al.*,1988). The recent studies observed significant rise in serum adenosine deaminase in a group of uraemic patients. The argued that this rise could have been the result of hepatic microsomal enzyme induction, process thought to accompany acute renal failure. (Yasuda *et al.*, 1996; Aldich & Blackburn, 2000 and Trota & Balis, 1977). It is important to know whether enhanced hepatic microsomal activity occurs in uraemia. It is also important to know whether the uraemia may cause an increase in serum adenosine deaminase activity and what the clinical significance of such arise participates in the purine metabolism (Kobayash *et al.* 1993; Nonan, 1981and Ungerereta, 1992). where it degrades either adenosine or 2-deoxy adenosine producing inosine or 2-deoxyinosine,respectively (Salih, 2004). A high level of (SADA) in serum of cancer patient, hepatitis, cirrhosis and infectious mononuclease has been reported (Kohlen, 1967). Investigation of ascetic fluid of tuberculosis revealed a high activity of adenosine deaminase enzyme (`Vorg *et al.*, 1989)also different disorders has been investigated and revealed elevation in SADA activity in secondary liver tumors (Porma, 1996).

## Materials &Methods

### 1-Collection of samples

Blood analysis is usually done on venous of capillary .Sixty samples of blood were throughout the estimation of Adenosine deaminase activity forty of these samples were of acute renal failure, while the other are healthy individuals. Five milliliters of blood has been collected and allowed standing at room temperature until it has been clotted. The separated serum about 2-3 mL. is centrifuged at 3000 rpm using T.5 centrifuge for removal of any suspended cell.

### 2-Chemicals

All chemicals used were of high analysis grade and parched from BDH, Fluka( Table1)

**Table (1):Chemicals**

| Substance | Concentration |
|---|---|
| Phosphate buffer | 60mmol/L pH 7.2 |
| Adenosine ,buffered (Substrate) | 13mmol/L |
| Ammonium sulphate standard | 100mg/L |
| Alkaline hypochlorite | 100gm/L |

### 3-Instruments

*Spectrophotometer supertonic 601
* philips pH meter
*Thermostatic water bath
*Centrifuge T-5

### 4-Practical

### A-Enzymatic activity assay

The protocol of Giusti and Galanti (1974) was adopted for the determination of SADA activity. The principles depend on liberation of ammonia by enzymatic deamination of adenosine. The activity was estimated using spectrophotometric analysis in which a colored complex is absorbed at 630 nm using series concentration of substrate fig(2).

### B-Determination of kinetic parameters (Km &V max)

The values of Km and V max were measured in acute renal failure case and healthy individuals. The same experimental protocol described in A was adopted and only difference is the use of different substrate concentration (0.25,0.35,0.5,0.75,1,1.5,2,3,4,and 5mM). The data obtained were examined for Km & V max by using following methods of plotting

1-Micheales-Mentin plot in which the rate of reaction (V) is plotted Vs [S]
2-Lineweaver-Burk plot. Reciprocal plot of 1/V Vs 1/[S] has been plotted

**C-Determination of optimum pH value**

Wide ranges of pH (5-12) has been used in order to determine the highest serum adenosine deaminase (S-ADA) activity at optimum pH value, the same protocol A was used

**D-Determination of optimum temperature and activation energies (Ea)**

Various incubation temperatures of (10, 20, 25,30,35,37,45,50 ,60ć) have been applied the same protocol as in A was used.

## Results and discussion

The activity of S-ADA in acute renal failure was estimated and compared with that of healthy individuals using Giusti and Galanti method .The results obtained showed significant elevation of S-ADA activity in uraemic patient (63.2±20 IU/L) in comparison with that of healthy individuals (28±9.3 IU/L) fig(1)

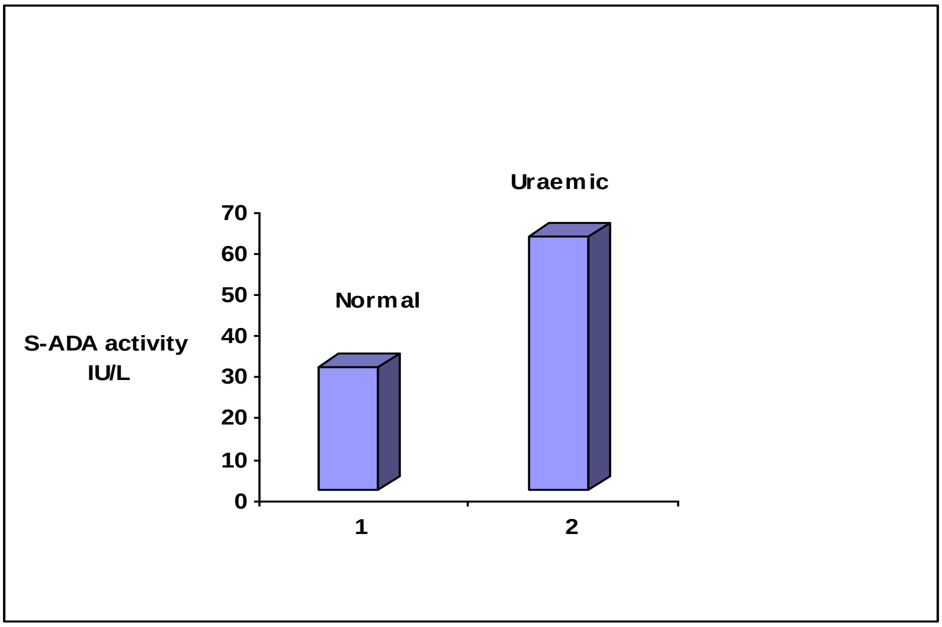


**Fig.(1): Serum Adenosine deaminase activity in normal and uremic serum**

S-ADA activity has been found in high levels in the myocardium, kidney, muscles and liver (Vicins *et al.*, 1991). Also S-ADA activity has been reported to be high in hepatitis, tuberculosis, toxoplasmosis, ricketosis, rheumatoid arthuritis and leukemia in human(Harvy *et al.*, 1978). Elevation of SADA activity can be explained to be due to the affection of liver tissue by renal failure and damage of liver cells resulting in the release of intracellular components into the blood, hence elevation its level in the blood (Brichman *et al.*, 1974). Table (2): shows the determined values of Km & Vmax in healthy individuals and uraemic patient

**Table (2):Physical parameters (Km&Vmax) of normal and ureamic SADA serum**

| Parameter | Normal serum | Ureamic serum |
|---|---|---|
| Km(Mm) | 1.5±0.2 4 | 0.99±0.34 |
| Vmax(IU/L) | 28±9.2IU/L | 63±20.1 IU/L |

the important of Km and V max values is that their level variations can be presumed to be a diagnostic marker for abnormal cases .This is due to the possessed affinity of the enzyme to catalysis. Fig.(2,3) also shows Km and V max values for healthy individuals and uraemic patient

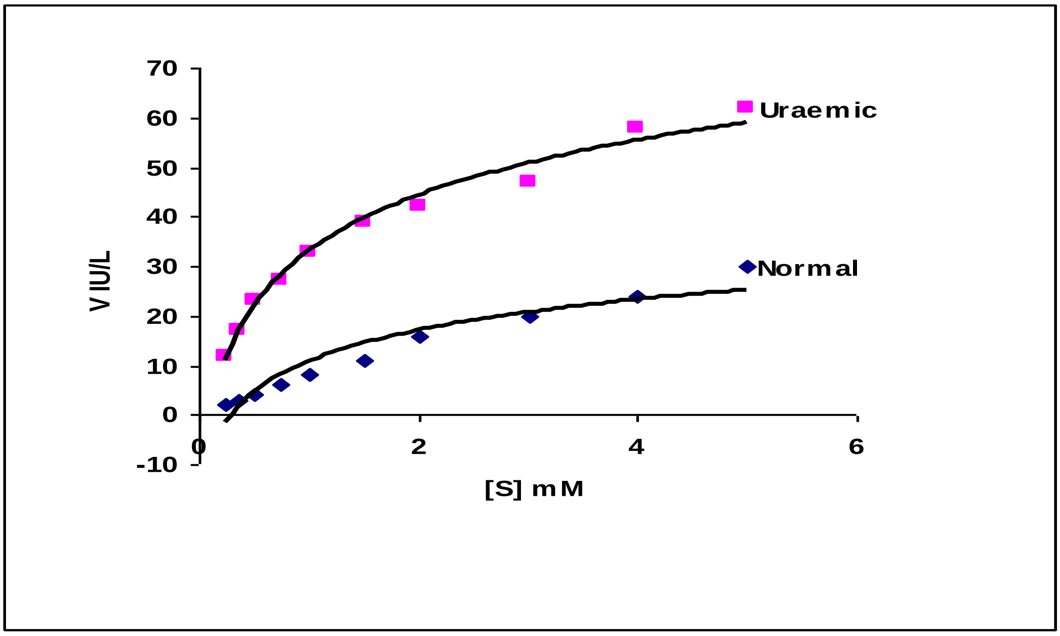


**Fig. (2): Michaelis-Menten plot for serum Adenosine deaminase activity in normal and uremic serum**

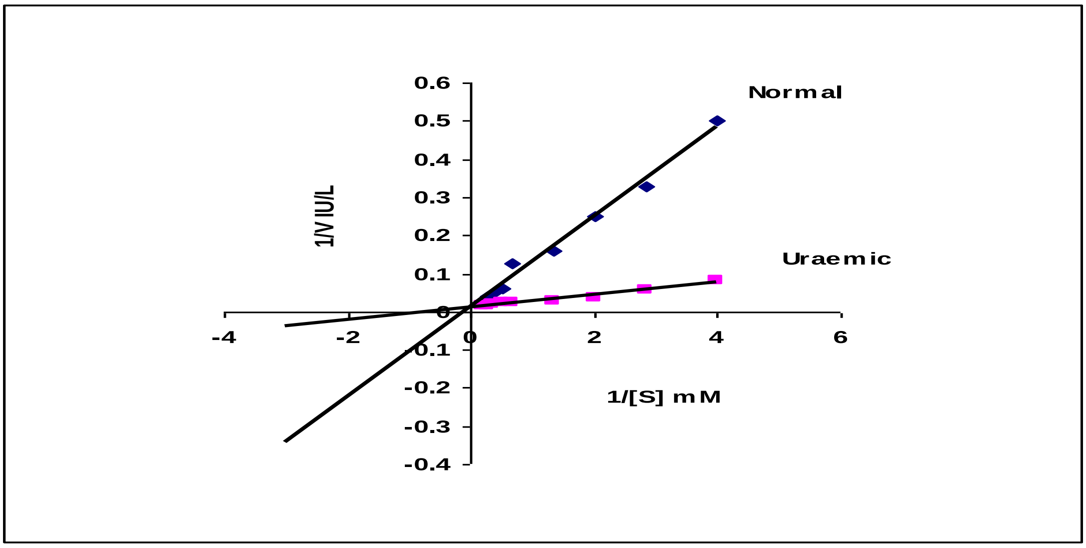


**Fig.(3): Lineweaver-Burk plot for normal and uremic serum.**

we note that there is notable decrease in Km value for renal failure S-ADA enzyme when compared with of healthy individuals S-ADA enzyme while the activity (V max) of S-ADA enzyme enhanced in acute renal failure (63.2±20IU/L) rather than in healthy individuals (28±9.2IU/L). From our results we can conclude that the affinity of the enzyme is greater in the case of acute renal failure to its substrate .This may be due to changes in the ratio of the isoenzymes in acute renal failure patient, or may be due to the changes in the structure on conformation of the enzyme (Moos-Movahed, 1974; Hitolgo *et al.*, 2001).The pH has an effect on the tertiary structure of the enzyme, and therefore on its activity, so that the enzyme may be irreversibly denaturated at extreme pH value. (Al-dahha, 1992) As it is clear in figure(4) that SADA enzyme from uraemic serum is highly sensitive to the changes in the pH when compared with that of healthy individual serum.

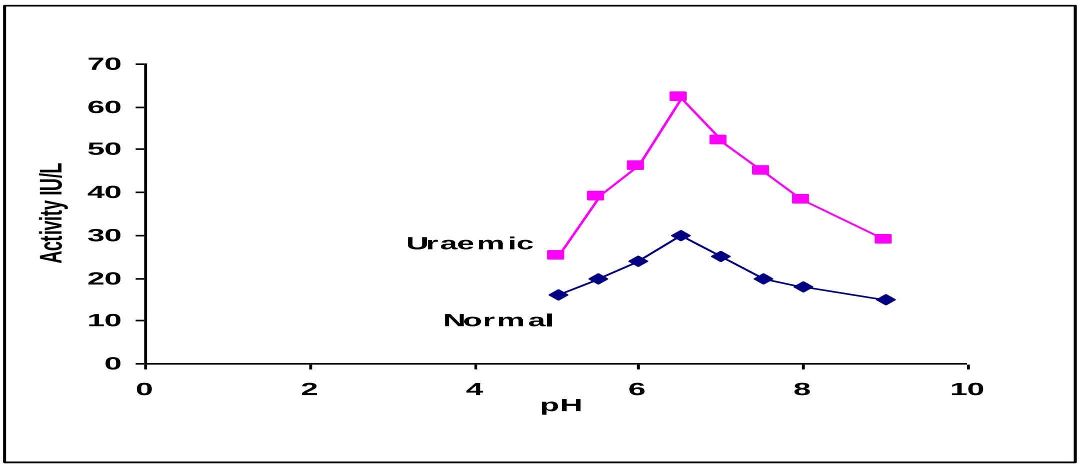


**Fig.(4): pH effect on normal and uremic serum**

Highest activity of S-ADA at pH 6.5 was found in both uraemic and normal serum. This can be explained to be due to alteration in the protein nature of the enzyme and exactly its active site, i.e amino acid content and their ionic state. Hence, alteration will occur in the enzyme free form or I the enzyme- adenosine complex of the transition state (Koldberg, 2001; Cooddly, 1971).

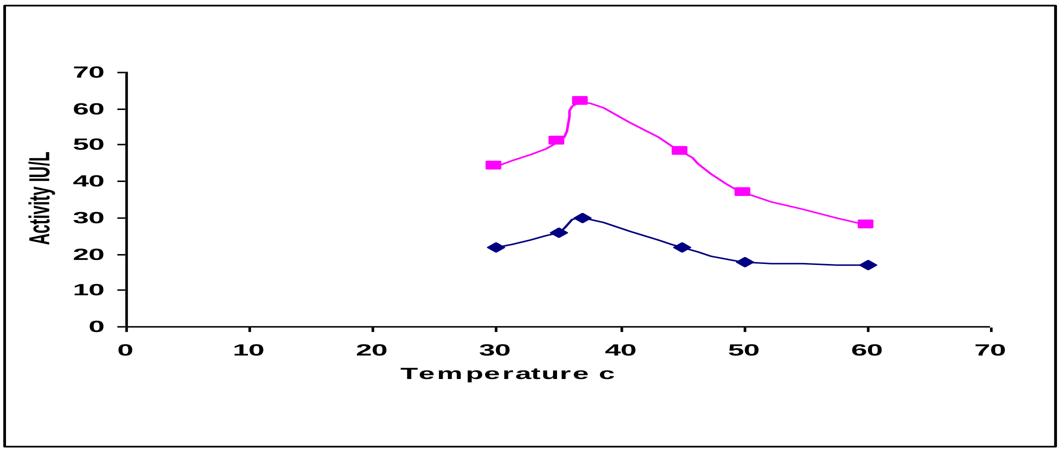


**Fig.(5): Effect of temperature on normal and uremic serum**

However from fig.(5)we note that, above and below optimum temperature , uraemic S-ADA enzyme is more heat stable than normal S-ADA enzyme.This may be due to the differences in the ratio of isoenzymes in the serum of uraemic patient because isoenzymes have different stability to heat.(Colombo *et al*., 2002). The tertiary structure of a enzyme is maintaine primarily by a large numbers of weak noncovalent bonds. If molecule absorbe too much energy the tertiary structure will disturb, and the enzyme be will denaturated, that loses its catalytic activity (Godberg and Fletcher, 1966).Activation energy (Ea*)was determined in both normal and uraemic S-ADA enzyme from Arrhenius plot I which logarithm of activity ploted against inversed absolute temperature. According to Arrhenius equation, aplot of Log.V Vs 1/T K was investigated as LogV=-_Ea/2.3 R .1 /T +LogA ------(Arrhrnius equation) Energy of activation has been estimated from the slop of the curve fig. (6)

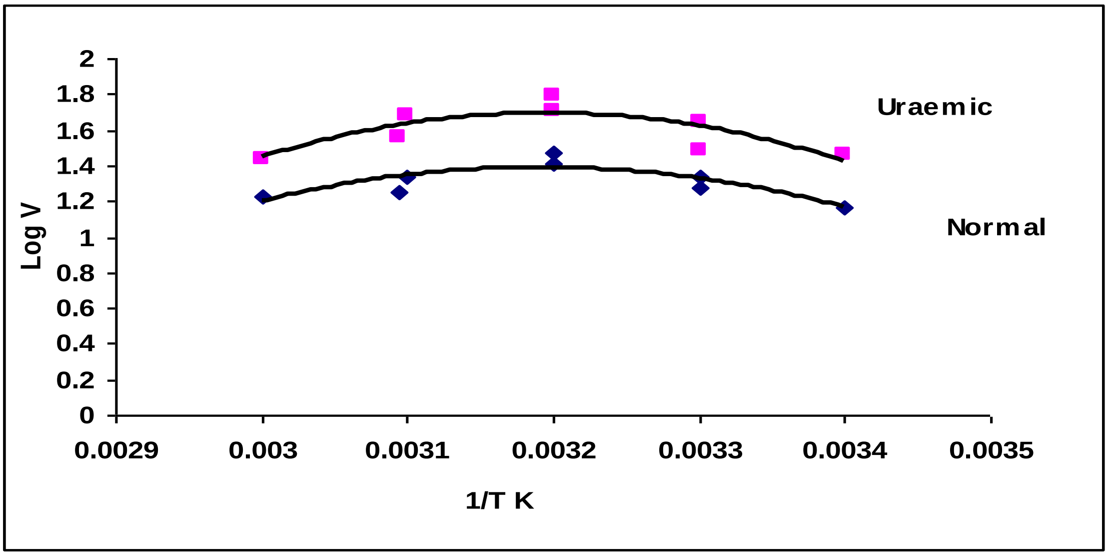


**Fig.(6): Arrhenius plofor both on normal and uremic serum**
**The activation energy of uraemic S-ADA enzyme is greater than that of normal S-ADA enzyme table (2)**

**Table (3): Activation energy for S.ADA in both Normal &ureamic serum**

| Enzyme | Activation energy Ea | |
|---|---|---|
| | Normal | Ureamic serum |
| ADA | 3415.5 | 6831 |

,uraemic S-ADA enzyme must obtain greater amount of energy in order to pass the energy barrier of the enzymatic reaction to produce products. This is a ppoint of interest suggesting that the enzyme concentration increased in the circulation causing an alteration in its activity. This may be due to the releasing of S-ADA enzyme from different sources such as liver and kidney (Ross, 1969; Mocana *et al*., 1983).

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# دراسة حركية لنشاط الاينوسين دى امينيز في المرضى المصابين بالفشل الكلوى

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## الخلاصة

قيست مستوى الإنزيم الاينوسن دى امينيز فى مصول المرضى المصابين بالفشل الكلوى الحاد وتم مقارنتها بالنتائج للأشخاص الصحاء حيث وجد بان مستوى في مصول المرضى يبلغ ارتفاعا معنويا وهو(٢٠±٦٣)هو بدلك أعلى من معدلاته فى مصول الاشخاص الاصحاء والبالغة (٢٨±٩) كما ابدى لاينوسن دى امينيزقمة نشاطهفى اس الهيدروجينى ٥و٦ودرجة حرارة٣٧ كدلك تطرقت الدراسة تقدير طاقةشيط لتكوين المعقد.