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Research Article

STUDY OF SERUM ADENOSINE DEAMINASE ACTIVITY (ADA) IN DIABETES MELLITUS WITH COMPLICATIONS

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ABSTRACT
Diabetes mellitus is a complex syndrome characterized by hyperglycemia, leading to vascular complications such as retinopathy, neuropathy and macrovascular disease like atherosclerosis. Patients with diabetes with complications and without complications are taken up for the study. The parameter serum Adenosine Deaminase, Activity (ADA) is considered, which is helpful in understanding the predisposing factors and the assessment of these patients to develop complications. The present study reveals that serum ADA is found to be raised in diabetic patients without complications while it is similar to control group with complications. This indicates that increased susceptibility of diabetes to develop a variety of bacterial and fungal infection may not be due to immune deficiency.

Key Words: *Serum Adenosine Deaminase Activity (ADA), Diabetic*

INTRODUCTION
Adenosine deaminase in lymphoid tissue might efficiently deaminase deoxy adenosine and prevents phosphorylation.

Galanti and Giusti Altivita (1968) and Gold Berg and Elis (1976) observed that Human serum ADA increases in acute viral hepatitis and active cirrhosis and only to a much lesser extent in other hepatic diseases. Goldblum *et al.,* (1978) demonstrates that increased serum ADA has been found in leukaemic patients and lymphocyte ADA levels can be considered a parameter of immune response Lymphocyte ADA activity is decreased in erythrocytes from patients with severe combined immunodeficiency, while heterozygous carrier of this autosomal recessive defect have half the normal enzyme activity.

Alan Taylor (1986) observed an increase in serum ADA activity in 18 untreated patients with active sarcoidosis and suggested with some reservation that its measurements might be useful for diagnosis of sarcoidosis. Singh *et al.,* (1981) reveals that the estimation of ADA activity will be of value in the diagnosis of tuberculous effusions. The high ADA activity in tuberculous effusions could be attributed to cell mediated immune reactions or to increased demands for energy. This high level may be useful in the pleural differential diagnosis of tuberculous from other pleural effusions. Delias *et al.,* (1987) studied ADA activity in acquired Immunodeficiency syndrome and reveals high ADA activity in these subjects. ADA activity in lymphocytes and erythrocytes as well as in serum, is absent in about 20 – 30% of children affected by a severe inherited T cell immune deficiency. Yasuhera and Nakamera (1987) determined the activity of serum ADA in patients who had various types of pneumonia or pulmonary tuberculosis. ADA activity in children with bacterial pneumonia showed a higher value than those of viral and mycoplasma pneumonia but a lower value than that of tuberculosis. The peak ADA activity was found on 5^{th} or 6^{th} disease day in bacterial pneumonia. The number of lymphocytes is predominant over that of neutrophils at this period Serum ADA in tuberculosis showed highest concentration than that of pneumonia. Increased serum ADA in tuberculosis seems to be influenced by activated T lymphocytes.

MATERIALS AND METHODS
The study was carried out in 25 normal adult patients between the age group of 30-55 years from outpatient & inpatient department of OHRC & Princess Esra Hospital, Hyderabad, these patients show no family history of diabetes they did not suffer from any complication.

20 patients with NIDDM of 5-7 years duration is studied there was no renal impairment as shown by urine examination, blood urea and serum creatinine.

18 patients is studied suffering from NIDDM of more than 10 years duration with complications like retinopathy or nephropathy or neuropathy blood samples were collected in dry bottles using EDTA as auticoagulent for estimation of blood sugar and serum creatinine adenosine deaminase are also estimated.

The following parameters are studied:

- Fasting plasma glucose
- Serum adenosine deaminase activity
- Serum creatinine

Estimation of Glucose

Method – Glucose Oxide – Peroxidase Method:

Principle

This is an enzymatic method employed in the clinical laboratory for the estimate of glucose.Glucose is oxidized by glucose oxidase to gluconic acid and H_2O_2 is liberated. The colorimetric indicator, quinonemine is generated from 4 – amino antipyrene and phenol by H_2O_2 under the catalytic action of peroxidase intensity of colour generated is directly proportional to glucose concentration.

Glucose + O_2 + H_2O

Gluconic acid + H_2O_2

2 H_2O_2 + 4 Aminoantiyrine + Phenol Quinonemine + 4 H_2O

Reagent 1: Phosphate buffer PH 7.0 = 100 mm 01/1

Phenol ……= 5mm 01/1

4 Aminoantipyrine…….=0.5mm 01/1

Glucose oxidase ……> 15 ku/1

Peroxidase …….> 1 ku/1

Reagent 2: Glucose standard 100 mg/1

Sample Material

Serum, Heparin, Plasma or Flouride – Plasma

The stability in serum and plasma is 1 day at 2-8 degree centrigrade, serum or plasma must be separated from erythrocyte within 60 minutes of collection.

Assay Procedure

Wave length	Hg 546mm 500-540mm
Light path	1 cm
Temperature	37 degree centigrade
Measurement	Against regent block

Reference Range

Serum / Plasma 70 – 110 mg/dl

- Raaboe, Terkildsen Tc on the enzymatic determination of blood glucose, scand and clin lab invest.
- Trinder P.Glucose oxidase – Peroxidase method. Ann clin Biochem 1964:6:24.

Serum Adenosine Deaminase Activity

Principle

Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form blue indophenols complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indo phenol complex formed is directly proportional to the amount of ADA present in the sample.

Adenosine + ADA ammonia + inosine

Ammonia + Pheno + Hypo chlorite

Alkaline Blue indophenols comples

Medium

Reference Values

Serum Plasma Pleural, Pericardial and ascetic Fluids	Normal suspect strong	<30 u/l 30 u – 40 u/l.
	Suspect positive	>40 u/l – 60 ul.
		>60 u/l.
CSF	Normal Positive	< 10 u/l
		> 10 u/l

Reagents

- L_1 – ADA – MTB Reagent – Buffer Reagent, ready to use.
- L_2 – ADA – MTB Reagent – Adenosine Reagent, ready to use.
- L_3 – ADA – MTB Reagent – Phenol Reagent.
- L_4 – ADA – MTB Reagent – Hypochlorite Reagent.
- S – ADA – MTB Standard ADA standard – ready to use.

Reagent Preparation

Reagents L_1 L_2 and standard are ready to use adenosine reagent (L_2) may be form crystals at 2-8^{oc} dissolve the same by gently warming (35 ^{oc} 50 ^{oc}) the reagent for some time before use both the phenol reagent (L_3) AND THE Hypochlorite reagent (L_3) need to be diluted 1:5 distilled water before use (1) part of reagent + 4 parts of distilled water).

Test Procedure

- Bring all reagent and samples to room temperature before use.
- Prepare the working phenol regent and working hypochlorite reagent.
- Set the spectrophotometer filter at 570 – 630 (Hg 578 to 623nm) at 37 ^{oc}
- Pipette into clean dry test tubes labeled blank (B) standard (S) sample.

Blank B (SB) and test (T) as follows:

Addition Sequence	**B (ml)**	**S (ml)**	**SB (ml)**	**T (ml)**
Buffer Reagent	0.20	0.20	-	-
Adenosine Reagent	-	-	0.20	0.20
Deionised water	0.20	-	-	-
Standard	-	0.20	-	-
Sample	-	-	-	0.20

5. Mix well and incubate at 37 ^{oc} for exactly 60 minutes and then add the following:

	B	**S**	**SB**	**T**
Working phenol reagent	0.20	0.20	-	-
Sample	-	-	0.20	0.20
Working hypchlorite Reagent	0.20	-	-	-

6. Mix well and incubate at 37 ^{oc} for 15 minutes at RT for 30 minutes.

7. Measure the absorbance of the blank (Abs B) standard (Abs S) sample blank (Abs SB) and test (Abs T) against distilled water.

Calculations

$$\text{Total ADA activity in u/l} = \frac{\text{Abs t} - \text{Abs SB}}{\text{Abs S} - \text{Abs B}} \times 50$$

The procedure is linear upto 150 u/l. If values exceed his limit dilute the sample with deionised water and repeat the assay.

- Jose L Banales *et al.,* (1991) Chest 99/2: 355.
- Imma ocana *et al.,* (1986) thorax 41:888-889.
- Diagnostic value of ADA and its isoenzymes in tuberculosis effusions, Dept. of internal medicine.
- Date of file Tulip Diagnostic (P) Ltd.

Estimation of Serum Creatinine

Determination of creatinine based on Jafe's kinetic method without deprotienization.

Principle

Creatinine forms a yellow orange compound in alkaline solution with picric acid. At a low concentration of picric acid as used in this method, precipitation of protein does not take place. As a result of rapid reaction between creatinine and picric acid, the secondary reactions do not cause interference.

Reagents

Reagents 1 Buffer solution
Reagents 2 picric Acid
Reagents 3 standard solution

Storage and Reagent Stability

The reagent stable till the date of expiry if stored at 15 – 25 ^{oc}.

Reagent Preparation

Pre-warm the reagents as well as sample Serum or Plasma and Urine Sample.

Reagent Start

Mix reagent 1 and reagent 2 in the ration of 1 + 1 (eg: 1 ml of buffer solution and 1 ml of picric acid solution) the mixing ration should be observed exactly.

Leave the monoreagent for atleast 10 min. at now temperature before using. The stability of the reaction solution is 5 hours at 15-20 ^{oc}.

Test Concentrations

Reagent	1	NaoH	313 mmol/1
		Phosphate	12.5mmol/l
Reagent	2	Picric acid	8.73mmol/l
Reagent	3	Creatinine standard	1.0mg/dl standard

Sample Material

Serum – Heparin – Plasma

Dilute Urine 1 + 99 with distilled water. The stability in serum and plasma is 7 days at 4 – 25 ^{oc} and at least 3 months at – 20 ^{oc} the stability in Urine in 2 days at 20 – 25 ^{oc} 6days at 4-8 ^{oc} and 6 months at 20 ^{oc} .

Assay Procedure

Wave Length	-	Hg 492mm (490 – 510nm)
Light Path	-	1 cm
Temperature	-	20 ^{oc} – 25 / 37 ^{oc}

Substrate Start: Sample / Std.

Sample / Standard	100 ul
Reagent 1	500 ul

Mix and incubate for 0 – 5 min, then add

Reagent 2	500 ul

Mix and read absorbance A_1, after 60 sec, read absorbance A_2 after further 120 sec.

Sample Start:	Sample / Standard
Sample / Standard	100 ul
Monoreagent	1000ul

Mix and read absorbance A_1 after 60 sec, read absorbance A_2 after further 120 sec.

Calculations

Serum / Plasma
Creatinine (mg/dl) = A Sample x Conc. Std. (mg/dl)
A Std.

Urine

Women = 7.3 – 21.4 mg/kg/d
Men = 8.7 – 24.6 mg/kg/d

RESULTS AND DISCUSSION

Results of study indicate that predisposition of diabetics to develop complications such as retinopathy nephropathy and the predisposition to infections in multifactorial.

Table 1: Serum Creatinine Levels in Various Study Groups

S. No	Group – I Normal Control	Group – II NIDDM without Complications	Group – III NIDDM with complications
1.	1	1.7	1.5
2.	0.6	1.7	1.6
3.	0.8	1.2	1.5
4.	0.6	1.6	6.5
5.	0.8	1.3	1.1
6.	0.7	1.3	3.6
7.	0.8	1.2	3.5
8.	0.7	1.5	2.6
9.	0.8	1.6	2.5
10.	0.9	1.5	2.2
11.	0.6	1.5	2.2
12.	1.0	1.1	2.3
13.	0.8	1.2	1.5
14.	0.6	1.1	1.9
15.	0.6	1.3	4.2
16.	0.8	1.2	1.4
17.	0.7	1.4	1.8
18.	0.753	1.318	2.818
19.	0.1328	0.3661	1.9340
20.	0.0322	0.0888	0.4691

Serum Creatinine

Source	DF	Sum of Squares	Mean of Square	F-Ration	Significance
Between group	2	42.18	21.09	15.74	<.01
Within group	48	64.62	1.34		
Total					

Fasting blood sugar levels are found to be raised in all patients who have already developed micro vascular complications control (72.45 $\pm$ 8.662) NIDDM without complications (159.00$\pm$43.865) NIDDM with complications (212.64$\pm$74.938) (Table 3).

Table 2: Adenosine Deaminase Levels in Various Study Groups

S. No	Group – I Normal Control	Group – II NIDDM without Complications	Group – III NIDDM with complications
1.	16	17.8	12.12
2.	13	13	12
3.	12	11	12
4.	15	14	16
5.	12	15	13
6.	13	20	15
7.	14	16	10
8.	16	21	14
9.	15	15	15
10.	14	15	12
11.	12	13	16
12.	12	14	15
13.	15	14	17
14.	14	16	10
15.	12	14	15
16.	12	11	15
17.	13	11	12
18.	16	17	14
19.	16	16	16
20.	14	13	11
MEAN	13.800	14.850	13.600
SD	1.5424	2.7198	2.1126
SE	0.3449	0.6082	0.4724

Serum creatinine is raised in chronic diabetic patients who already developed nephropathy (Table 1). In these patients the blood glucose is also raised (Table 3). Different clinical and biochemical studies also show that occurrence of diabetic complications is more in patients with poor glycemic control.

Serum ADA activity is normal in diabetic with retinopathy and nephropathy however the enzyme activity was found to be slightly higher in diabetics without these complications (Table 2). The present study suggest that degree of hyperglycemia related to ADA increased adenosine deaminase level reflecting increased 'T' cell function in diabetics without complications as retinopathy or nephropathy may be due to autoimmune reaction against modification glycated proteins. Serum ADA levels is found to be raised in diabetic patients without complications while it was similar to control group with above complications this indicate that increased susceptibility of diabetics to develop a variety of bacterial and fungal infection may not be due to immune deficiency (Table 2). It has been conclusively shown that strict control of blood sugar levels reduces the risk of developing complications like neuropathy retinopathy and prevention of CAD.

Serum Creatinine

Source	DF	Sum of Squares	Mean of Square	F-Ration	Significance
Between group	2	17.73	8.865	2.94	ns
Within group	57	-171.7	3.01		
Total	59				

Table 3: Fasting Plasma Glucose (mg/dI)

S. No	Group – I Normal Control	Group – II NIDDM without Complications	Group – III NIDDM with complications
1.	76	150	280
2.	60	170	132
3.	76	180	230
4.	60	180	285
5.	68	170	192
6.	64	150	286
7.	78	250	260
8.	72	140	78
9.	90	80	286
10.	80	90	118
11.	92	80	187
12.	76	180	250
13.	64	148	364
14.	72	140	75
15.	60	176	275
16.	76	180	240
17.	72	132	118
18.	68	172	180
19.	68	140	185
20.	74	250	238
21.	68	140	189
22.	80	200	230
MEAN	72.45	159.00	212.64
SD	8.662	43.865	74.938
SE	1.847	9.352	15.977

Serum Creatinine

Source	DF	Sum of Squares	Mean of Square	F-Ration	Significance
Between group	2	127015.23	6250761.50	51.43	P<0.01
Within group	57	70382.00	123477.20		
Total					

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