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RESEARCH ARTICLE

ROLE OF SERUM ADENOSINE DEAMINASE LEVEL IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Introduction: Diabetes mellitus is a cluster of abnormal metabolic disorder having common features of hyperglycaemia with a state of increased free radical activity. Chronic hyperglycemic status favours auto-oxidation and the formation of advanced glycation end products. Adenosine deaminase (ADA) is considered as a good marker of cell mediated immunity. Increased ADA activity in diabetic individuals couldbe due to altered insulin related T-lymphocyte function.

Methodology: This study was done as case control study among 60 cases and 40 controls. The cases were further divided into two groups based on HbA1c levels. All the parameters like FBS, PPBS, HbA1c and ADA were measured.

Results: In our study, ADA levels was significantly high in controlled diabetics (group II with HbA1C < 7) and was much higher in Uncontrolled diabetics (group III with HbA1 C > 7) compared to healthy controls (group I). Comparison of the parameters (FBS, PPBS, HbA1 C, ADA) between the 3 group was done using Student t test and was statistically significant. Pearson's coefficient correlation was done between ADA and HbA1 C and found a positive correlation between them and had a statistical significance.

Conclusion: This study indicates that ADA raises with the extent of severity of type 2 diabetes. Positive correlation of ADA with HbA1 C provides the information that ADA can be considered to reflect the glycemic status of the individual.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine metabolic disorder responsible for increased morbidity, mortality and complications. The burden of DM is being evidenced recently in large numbers, much to the advent of large screening programmes and lifestyle changes. It consists of the following pathological changes:

- Resistance to the action of insulin in peripheral tissues, particularly muscle and adipose tissue;
- Decreased insulin secretion
- Increased glucose production by the liver.

Insulin resistance is decreased biological response to normal concentrations of circulating insulin. It plays a central role in pathophysiology of type 2 Diabetes.

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Diabetes mellitus is associated with oxidative stress which occurs as a result of imbalance between pro-oxidants and antioxidants. Monitoring glycemic control is an essential component of diabetic care (Thakur, 2009). The onset of complications is linked to the accumulation of glycation adducts in tissue proteins, any analytical method that serves as an index of the extent of glycation should clearly be used to guide therapy in diabetes. The core of the issue is glycemic control. Amongst the various markers of glycemic control, glycated hemoglobin has now been established as the most reliable (Chandalia, 2002). Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses and inappropriate T-lymphocyte function, which is vital in diabetes and has a link with insulin defect (Chang, 1995). Adenosine deaminase, an enzyme distributed in the human tissues, was considered as good marker of cell mediated immunity. It plays a crucial role in lymphocyte proliferation and differentiation9 and shows its highest activity in T-lymphocytes (Sullivan, 1997). Adenosine deaminase (ADA) is an enzyme of purine metabolism. It acts on adenosine and

other adenosine nucleoside analogues and catalyze its hydrolytic cleavage into inosine and ammonia. It is a cytosolic enzyme, which has been the object of considerableinterest. Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium. Adenosine modulates the action of insulin on various tissues differently. Concentration of Adenosine in tissues is affected by ADA level (Warrier, 1995). Adenosine deaminase has been previously reported to be a marker for insulin function (Kurtul, 2004; Hoshino *et al*., 1994). But its connection with the immune system was not yet established in diabetic subjects. Even though there are some reports available on adenosine deaminase levels in diabetic subjects, these are all inconclusive and controversial (Kurtul, 2004) Since a relationship exists between adenosine deaminase and cell mediated immunity (Baghanha *et al*., 1990), we have undertaken this study to determine its activity in serum and understand its importance in the immunopathogenesis of type 2 diabetes mellitus.

Objectives

- To estimate the level of Serum Adenosine deaminase (ADA) among the patients with type 2 diabetes mellitus through a case control study.
- To correlate Adenosine deaminase with glycemic control (HbA1C).

METHODOLOGY

Study Setting

The study was conducted as a case control study among Type 2 diabetic patients attending diabetic outpatient department and healthy non diabetic controls who came for routine check - up at our tertiary care centre in Chennai between December 2014 and May 2015.

Study Population

Based on intensive literature review, a study done by Amandeep Kaur *et al*. reported the mean±S.D ADA levels among diabetics and non diabetics to be 30.4±10.4 and 17.3±7.3U/L. At 95% confidence limits and 1.28 power, the estimated sample size was 10.44 subjects in each group. In this study, 40 controls (group I) and 60 cases were included in the study. The cases were divided into two groups, one with HbA1C <7% (group II) and one group with HbA1C >7% (group III).

Ethical approval and informed consent

Approval from the Institutional Ethics Committee was obtained and informed consent was obtained from all the participants prior to data collection.

Inclusion Criteria: For cases

- Clinically diagnosed cases of type 2 diabetes mellitus are included in the study.
- Cases are in the age group of 30-60 yrs including both male and female.

For controls

Age, Sex, BMI matched healthy individuals as controls.

Exclusion Criteria

- Diabetic patients who have symptoms of obvious complications of diabetes.
- H/o rheumatoid arthritis, viral hepatitis, psoriasis, tuberculosis.
- Diabetics on insulin therapy.
- Pregnant women

Tools for Data collection

Age, gender, height, weight, DM duration, general history and medications were recorded. Blood samples were collected following overnight fasting. Samples were collected by 5 ml of venous blood was drawn from each volunteer using a disposable vacutainer system in fasting condition. Post prandial (2 hour) sample collected in fluoride vacutainer for PPBS estimation. Serum or plasma separated within half an hour and stored at 2-8° C till analysis was done.

Estimation of Fasting plasma sugar

Method: GOD/POD: enzymatic photometric Method.

Principle

The principle is determination of glucose after enzymatic oxidation by Glucoseoxidase. The colorimetric indicator is quinoneimine, which is generated from 4 -aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder reaction).

GOD: Glucose + O2 ► Gluconic acid + H2O2 POD: 2H2O2+4-aminoantipyrine+phenol ► Quinoneimine + 4H2O

Assay procedure

End Point Procedure; Wavelength 505nm; Optical path-1 cm; Temperature 20-25°C/37°C. Measurement- Against reagent blank; Sample or standard-10ul; Reagent -1000 ul Mix, incubate 15 minutes, at 20 -25°C or 10 minutes, at 37°C. Read the absorbance against the blank within 60 minutes.

Calculation: Glucose in mg% = Abs. of (T) X (Std. Conc) Abs. of (S)

Estimation of HbA1C by Ion Exchange Resin method

Principle of the method

When there is hyperglycaemia, proteins in the body undergo non enzymatic glycation. Glucose first forms a labile Schiff's base with the N -terminal amino groups of the proteins. Following this there is Amadori rearrangement which results in the formation of stable ketamines. In this assay, whole blood is mixed with a lysing agent containing a detergent and a high concentration of borate ions. This eliminates the labile Schiff's base. The hemolysat e is mixed with weak cation exchanged resin to allow the binding of the labile fraction to the resin. By means of a separator, the resin is separated from the buffer solution containing unbound HbA1c. The percentage of HbA1 c is determined by measuring the absorbance of HbA1c fraction and total haemoglobin at 415 nm.

Assay procedure

Wavelength -415 nm; Optical path - 1 cm; Room temp

Step 1:

- 250ul of lysing reagent pipetted into required number of labelled tubes for different samples.
- 50 ul of well mixed whole blood sample pipetted into the appropriately labelled tube and mixed well.
- Incubated for 5 minutes at room temperature to allow complete lysis of RBC.

Step 2:

- Pre-pipetted ion exchange resin tubes were taken and cap removed
- 100 ul hemolysate reagent obtained in step 1 was added into the appropriately labelled ion exchange tubes.
- A resin separator was inserted into each tube so that the rubber sleeve was approximately 1 cm above the liquid level of the resin suspension. The tubes were mixed on a vortex mixer continuously for 5 minutes.
- The resin allowed to settle, then the resin separator was pushed into the tubes until the resin was firmly packed.
- The supernatant poured into a cuvette, and the absorbance read at 41 5 nm against blank.

Step 3:

- 5 ml distilled water dispensed into labelled tubes.
- 20 ul hemolysate obtained in step 1 was added into the appropriately labelled tubes. Mixed well and absorbance read at 41 5 nm against distilled water.

Calculation: %HbA1c (%GHb) =AGHb x Factor ATHb

Estimation of Adenosine deaminase by colorimetric method

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, especially high in T lymphocytes. Elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma.

Assay Principle

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted with N - Ethyl - N - (2 - hydroxy - 3 - sulfopropyl) - 3 -methylaniline (EHSPT) and 4-aminoantipyrine (4- AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

ADA: Adenosine + H_2O>Inosine + NH_3
Inosine + Pi> Hypoxanthine + Ribose-1- phosphate

XOD Hypoxanthine + $2H_2O_2$ Uric acid + $2H_2O_2$
POD H_2O_2 + 4-AA + EHSPT

(X max 556nm) *• $2H_2O$ + Quinone dye One unit of ADA is defined as the amount of ADA that generates one umole of inosine from adenosine per min at 37°C.

Reagent 1

Tris HCl, pH 8.0 - 50 mM, 4-AA - 2 mM, PNP - 0.1 U/mL, XOD - 0.2 U/Ml, Peroxidase - 0.6 U/mL and Stabilizers

Reagent 2

Tris-HCl, pH 4.0 - 50 mM, Adenosine 1 0 mM, EHSPT 2 mM

ADA Control

Adenosine deaminase (bovine liver) and BSAADA Control Reagent Preparation Liquid is a two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics). ADA control and calibrator are in lyophilized form, and need to be reconstituted with 1.0 mL of DI water before use. The reconstituted ADA controls and calibrator are stable for 1 week at 2-8°C. Control and calibrator sold separately.

Assay procedure

R1: 180 ML Sample: & pL R2:90 |iL 550 rtm| 37»C|/ / 0 3 mln 6 mln 11 min A1 A2. Calculations ADA Activity in U/L = A AT / AAC X Concentration of Calibrator A AT - mean absorbance of the test A AC - mean absorbance of the calibrator Linearity: 250 IU/L Measuring Wavelength 546 nm.

Data analysis

Data was entered and analysed using SPSS software ver.21. Mean and standard deviation was calculated for all the glycemic parameters. Independent sample t test was used to analyse the statistical significance of the parameters between the groups. Correlation coefficient was computed between ADA and HbA1c.

RESULTS

The study population comprised of a total of 100 individuals. All the study individuals were in the age group between 30 and 60 years. Mean ± S.D age of study participants were 49.28 ± 8.96 in Group I, 52.07 ± 9.13 in Group II and 47.27 ± 8.41 in Group III.

Table 1. Background characteristics

S. No	Characteristics	GroupI Mean ± S.D	Group II Mean ± S.D	Group III Mean ± S.D
1	Age	49.28 ±8.96	52.07 ± 9.13	47.27 ± 8.41
2	FBS	90.82±10.23	112 ± 4.93	142.07 ± 12.59
3	PPBS	140 ± 7.26	148.93 ± 6.26	192.60 ± 10.99
4	HBA1C	4.96 ± 0.60	6.48 ± 0.34	8.87± 1.20
5	ADA	35 ± 4.50	48.30 ± 2.97	57.30 ± 3.34

Among 100 participants in the study 23 were male and 17 were female participants in group I, 16 male and 14 female in Group II and 17 male and 13 female participants in Group III.

Table 2. Association of parameters between Group I & II

S. No	Parameters	Mean Difference	t- value	Df	p value
1	FBS	-0.35	-0.084	28	0.934
2	PPBS	-0.08	-0.03	28	0.974
3	HbA1C	0.06	0.249	28	0.805
4	ADA	-1.82	-1.47	28	0.153

In this study of 100 participants 40 healthy controls (Group I) were age, sex matched healthy individuals. Among 60 Diabetic individuals, one half were in group II belonging to have the HbA1C level < 7 and the other half were in group III belonging to have HbA1 C > 7. Background characteristics of study participants were given in table 1. Majority of the study participants in all the three groups were males. Table 2 gives the association of parameters between Group I and Group II. The results clearly shows that p > 0.05 for all parameters in Group I & II, which indicated that there is no significant association between the parameters in both groups. Table 3 gives the association of parameters between Group II and Group III. The results clearly shows that P > 0.05 for all parameters in Group II & III, which indicated that there is no significant association between the parameters in both groups

Table 3. Association of parameters between Group II & III

S. No	Parameters	Mean Difference	T value	Df	p value
1	FBS	-1.51	-0.32	28	0.751
2	PPBS	-1.42	-0.347	28	0.731
3	HbA1C	-0.26	-0.564	28	0.577
4	ADA	-1.82	-1.47	28	0.153

Table 4 gives the association of parameters between Group I and Group III. Here FBS has a significant association in Group I and Group III. And there is no significance difference found between PPBS, HbA1C and ADA between Group I and Group III.

Table 4. Association of parameters between Group I & III

S. No	Parameters	Mean Difference	T value	Df	P value
1	FBS	10.03	2.32	28	0.028
2	PPBS	3.50	0.85	28	0.42
3	HbA1C	0.73	1.728	28	0.095
4	ADA	1.61	1.31	28	0.235

Table 5. Correlation between HbA1c and ADA for the groups

S. No	Group	ADA & HbA1c	p value
1	I (N=40)	0.297	0.063
2	II(N=30)	0.370	0.044
3	III(N=30)	0.672	0.0001

Table 5 shows that Pearson's correlation of ADA levels of Group I, study group II and Study Group III with HbA1C are r=0.297, r = 0.37 and r=0.672 respectively. Both the correlations are statistically significant. Correlation coefficient(r) of Group II is significant at the 0.05 level (2-tailed). Correlation coefficient(r) of Group III is significant at the 0. 01 level (2-tailed).

DISCUSSION

This study was done among Type 2 Diabetic patients and Age, sex matched healthy individuals were taken as controls. Among the two study groups and the control group, the biochemical parameters including Fasting plasma sugar (FPS), Post prandial blood sugar (PPBS), Glycated haemoglobin (HbA1C) were performed. The blood glucose levels & glycosylated haemoglobin (glycemic marker) are monitored to estimate the glycemic status of the patient. A statistically significant difference was observed with all the parameters between the diabetic and non diabetic groups. In the view of assessing the glycemic level in diabetics with the help of ADA levels, ADA levels in all three groups were compared with HbA1C levels. Pearson's correlation of ADA levels of Group I, study group II and Study Group III with HbA1C are r=0.297, r = 0.37 and r=0. 672 respectively. Both the correlations are statistically significant. Correation coefficient(r) of Group II is significant at the 0. 05 level (2-tailed). Correlation coefficient(r) of Group III is significant at the 0.01 level (2-tailed). Therefore, a positive correlation was observed between ADA and HbA1C levels.

Adenosine deaminase (ADA) acts on adenosine and several other adenosine nucleoside analogues. Increased adenosine activity mimics the activity of insulin on glucose and lipid metabolism in adipose tissue. Also, ADA is considered to be a marker of T cell activation and a producer of reactive oxygen species (ROS). Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses and inappropriate T-lymphocyte function, which is vital in this pathogenic condition, has a link with insulin defect. Adenosine modulates insulin action on various tissues differently and its concentration in tissues is affected by ADA levels. Adenosine potentiated insulin and contraction stimulated glucose transport in skeletal muscles by enhancing the increase in GLUT-4 at the cell surface. This raised the possibility that decreased adenosine production or action due to raised ADA could play a causative role in insulin resistance. Adenosine exerts a protective role by inhibiting lipolysis. ADA inactivate adenosine, hence activates lipolysis causing increased cAMP levels. This elevation of free fatty acids causing dysregulated fat metabolism leads to further subsequent development of type 2 Diabetes. Various studies show altered Adenosine deaminase level in type 2 DM. Most of them showed increased adenosine deaminase activity in type 2 diabetes mellituspatients. Kurtul N *et al* have shown increased level of serum ADA activity in type 2 DM patients and its correlation with HbA1 c and suggested that ADA is an important enzyme for modulating the bioactivity of insulin.(6) Also suggest that ADA play important role in insulin effect and glycemic control. Increased activity of ADA might be marker for insulin. Hoshino T. *et al* also suggested that mean serum level of ADA1 and ADA2 level is high in NIDDM (noninsulin dependent diabetes mellitus) and IDDM (Insulin dependent diabetes mellitus) than healthy donor (higher in NIDDM than IDDM).(7) ADA2 activity in the poorly controlled NIDDM patients directly correlated with the HbA1c level.

Ogbu *et al*. has studied raised ADA activities in obesity which may be due to insulin resistance or increased secretion of adenosine.(10) M Shivaprakash *et al* observed significant increase in adenosine deaminase activity in diabetic subjects and hypothesizes that increased ADA activity may be due to altered immunity.(5) Therefore, ADA may serve as an immunoenzyme marker in the aetiopathology of type 2 DM. Anjali C. Warrier *et al* has shown increased ADA activity and its correlation with hyperglycemia (glycated hemoglobin) and lipid peroxidation in DM patients(11). They suggested that decreased tissue adenosine levels is due to increase in ADA

activity, is related to the severity of hyperglycemia and lipid peroxidation in diabetes mellitus. Gitanjali G, *et al* reported elevated level of serum ADA activity in DM type 2 patient and correlated it with markers of lipid peroxidation. They concluded that hyperglycemia aggravates oxidative stress, as well as increased levels of adenosine deaminase in diabetes, which plays an important role in DM, which may be because of local insulin resistance in the target organs and also because of the increased production of free radicals and oxidative stress. Our study data coincides with the previous literatures and estimates that Adenosine deaminase (ADA) levels were significantly high in type II diabetics than healthy controls.

Conclusion

This study results clearly shows that Adenosine deaminase (ADA) levels are increased in type 2 diabetics and positive correlation of ADA with glycemic control conveys that ADA may serve as a prognostic factor in type II diabetes mellitus. ADA, being an important enzyme for modulating the bioactivity of insulin and its essential role in the effect of insulin and glycemic control, it may also serve as a tool in assessing the extent of oxidative stress. All these features of ADA provides evidence to suggest ADA as a glycemic marker of type II diabetes. Hence, by analysing ADA levels in diabetics, glycemic control and insulin resistance can be assessed. Raised ADA levels can be an early indicator of progressive diabetic change insisting to initiate supportive therapy and preventive measures for the development of diabetic complication and thereby improving the outcome of the disease.

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