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RESEARCH ARTICLE
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STUDY OF SERUM ADENOSINE DEAMINASE LEVEL IN TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH GLYCEMIC CONTROL

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Abstract

Background: Diabetes mellitus (DM) is a chronic endocrine metabolic disorder but largely preventable non communicable disease which is responsible for millions of deaths annually, debilitating complications, and incalculable human misery.

Objective: To estimate the level of Serum Adenosine deaminase (ADA) among the patients with type 2 diabetes mellitus through a case control study and to evaluate the correlation of Adenosine deaminase with glycemic control (HbA_{1C}).

Methods: The study was conducted in a sample of 60 Type 2 diabetic patients attending diabetic outpatient department and 40 healthy non diabetic individuals who came for routine check -up at Sree Balaji Medical College & Hospital.

Results: ADA levels was significantly high in controlled diabetics (group II with HbA1C < 7) and was much higher in Uncontrolled diabetics (group III with HbA1C > 7) compared to healthy controls (group I). Comparison of the parameters (FBS, PPBS, HbA1C, ADA) between the 3 group was done using Student t test and was statistically significant. Pearson's coefficient correlation was done between ADA and HbA1C and found a positive correlation between them and had a statistical significance. This indicates that ADA raises with the extent of severity of type 2 diabetes. Positive correlation of ADA with HbA1C provides the information that ADA can be considered to reflect the glycemic status of the individual.

Conclusions: 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

Keywords: Serum Adenosine Deaminase Level, Type 2 Diabetes Mellitus, Correlation, Glycemic Control

Introduction

Insulin resistance is decreased biological response to normal concentrations of circulating insulin. It plays a central role in pathophysiology of type 2 Diabetes. Diabetes mellitus is associated with oxidative stress which occurs as a result of imbalance between pro-oxidants and antioxidants [1-3].
Assessing glycemia in diabetes has always been a challenge. Monitoring glycemic control is an essential component of diabetic care[4]. Complications occurrence 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[5].

Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses[6] and inappropriate T-lymphocyte function, which is vital in diabetes and has a link with insulin defect [7]. Adenosine deaminase, an enzyme distributed in the human tissues, was considered as good marker of cell mediated immunity[8]. It plays a crucial role in lymphocyte proliferation and differentiation[9] and shows its highest activity in T- lymphocytes[10].

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 considerable interest. 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[11].

Adenosine deaminase has been previously reported tobe a marker for insulin function[12, 13]. But its connectionwith the immune system was not yet established indiabetic subjects. Even though there are some reports available on adenosine deaminase levels in diabetic subjects, these are all inconclusive and controversial[12].Since a relationship exists between adenosine deaminase and cell mediated immunity[14] , we have undertaken this study to determine its activity in serum and understand its importance in the immunopathogenesis of type 2 diabetes mellitus.

Materials and Methods: The study was conducted in a sample of 60 Type 2 diabetic patients attending diabetic outpatient department and 40 healthy non diabetic individuals who came for routine check -up at Sree Balaji Medical College & Hospital.
Study individuals were divided into 3 groups.

Group I - comprised of 40 normal healthy individuals both males and females in the age group of 30-60 years from the general population who volunteered for getting included in the present study.
Group II- comprised of 30 patients of Type 2 Diabetes Mellitus including both males &females in the age group of 30-60 years on oral hypoglycaemic drugs with HbA_{1c}<7 %.
Group III - comprised of 30 patients of Type 2 Diabetes Mellitus including both male and female in the age group of 30-60 years on oral hypoglycaemic drugs with HbA1c>7 %.

This study was conducted between December 2014 and May 2015. The research and ethical committee of the university approved the study protocol. All participants were provided with written informed consent before enrolment in the study. Age, gender, height, weight, DM duration, general history and medications were recorded. Blood samples were collected following overnight fasting.

Method of Sample collection:
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.
Parameters assessed were: FBS (fasting blood sugar), PPBS (post prandial blood sugar), HbA1C (glycated haemoglobin), ADA (adenosine deaminase).

Inclusion Criteria:
For cases -
1.Clinically diagnosed cases of type 2 diabetes mellitus are included in the study.
2. 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:
i) Diabetic patients who have symptoms of obvious complications of diabetes.
ii) H/o rheumatoid arthritis, viral hepatitis, psoriasis, tuberculosis.
iii) Diabetics on insulin therapy.
iv) Pregnant women

Results:
The study population comprised of a total of 100 individuals, Of this 60 were Diabetic study individuals and 40 were healthy controls. All the study individuals were in the age group between 30 and 60 years. 40 healthy controls (Group I) were age, sex matched healthy individuals.
Of this 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 HbA1C > 7.
All the biochemical study parameters were analysed using Statistical Product and Service Solutions (SPSS) 17 software. Statistical tests used were Descriptives, Student t test & Pearson's Correlation. *P < .05 is significant, **P < .001 is strongly significant

Table 1- Descriptive statistics for group I:

		AGE	SEX	FBS	PPBS	HbA_{1C}	ADA
N	Valid	40	40	40	40	40	40
	Missing	0	0	0	0	0	0
Mean		49.2750		90.8250	140.0000	4.9675	35.0000
Std. Deviation		8.95570		10.23791	7.26071	.60272	4.50071

Table 2 - Descriptive statistics for group II:

		AGE	SEX	FBS	PPBS	HbA_{1C}	ADA
N	Valid	30	30	30	30	30	30
	Missing	0	0	0	0	0	0
Mean		52.0667		112.0000	148.9333	6.4767	48.3000
Std. Deviation		9.13098		4.92705	6.25842	.34209	2.97287

Table 3 - Descriptive statistics for group III:

		AGE	SEX	FBS	PPBS	HbA_{1C}	ADA
N	Valid	30	30	30	30	30	30
	Missing	0	0	0	0	0	0
Mean		47.2667		142.0667	192.6000	8.8633	57.3000
Std. Deviation		8.40744		12.58607	10.99404	1.20129	3.34406

Figure 1: Bar diagram depicting Comparision of FBS between group I, II, III

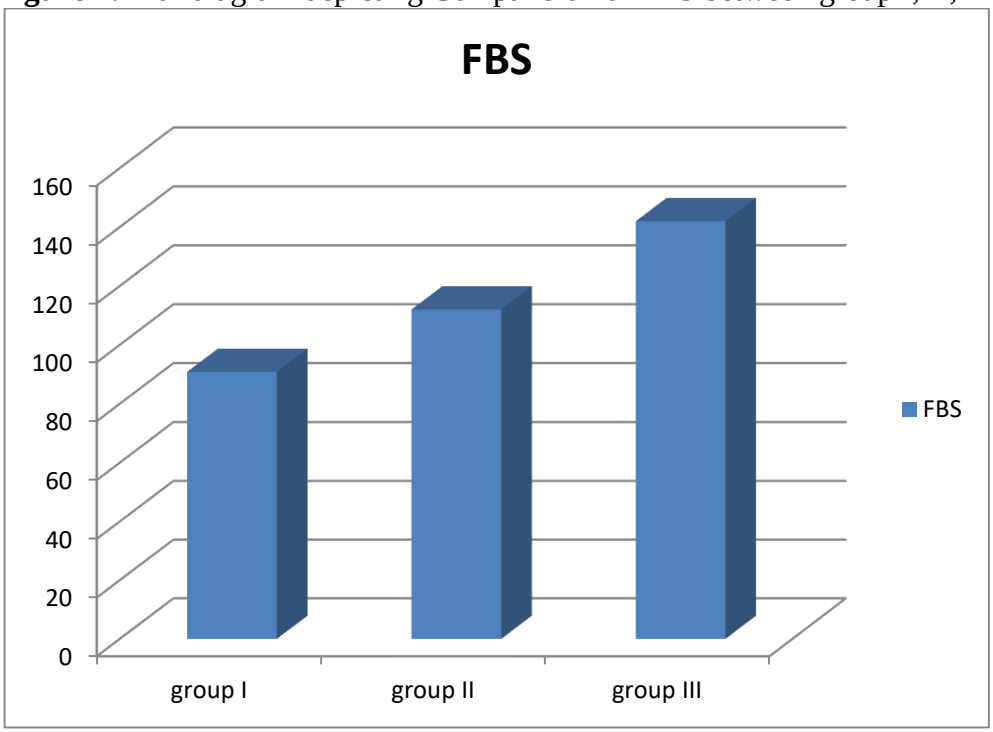


Figure 1: The values are expressed in Mean± SD. Student t test (two tailed) has been used to find the significance. The FBS values in group I , group II , group III are 90.8 ±10.23 , 112 ±4.92 and 142.06 ± 12.5 respectively . The levels in diabetics are higher than healthy controls and the difference is strongly significant (P<0.02).

Figure 2: Bar diagram depicting Comparision of PPBS between group I, II, III

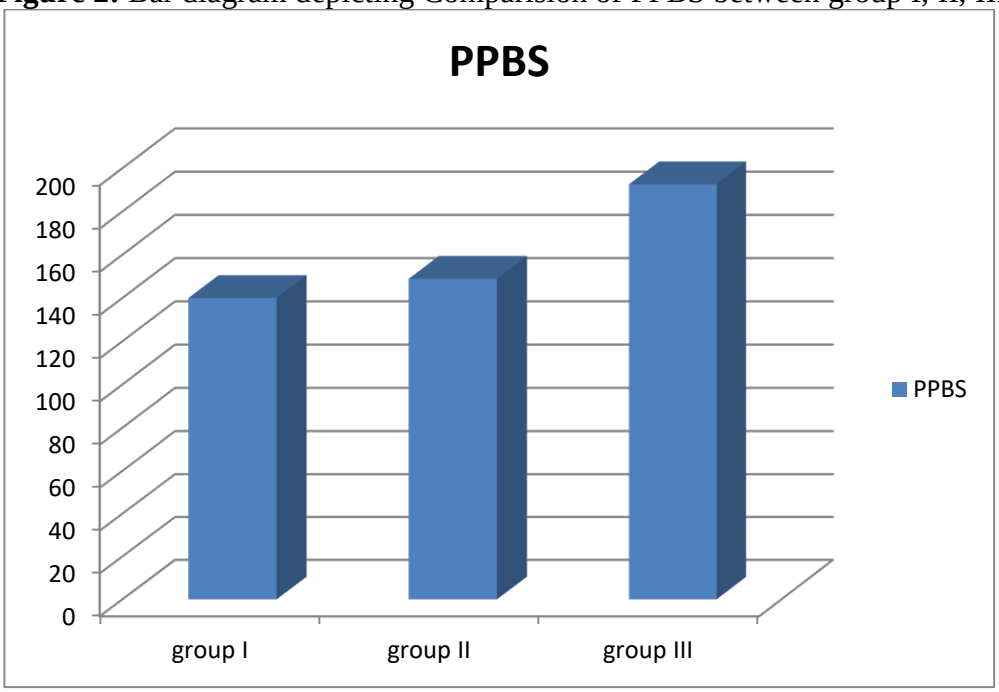


Figure 2 showed the comparison of PPBS and t test is done to estimate its significance. The PPBS values in group I, group II, group III are 140 ±7.26, 148 ± 6.25 and 192.6 ± 10.9 respectively. The levels in diabetics are higher than healthy controls and the difference is strongly significant (P<0.04).

Figure 3: The HbA1C values in group I, group II, group III are 4.96 ± 0.6, 6.47± 0.34 and 8.86 ± 1.2 respectively. Student t test (two tailed) has been used to find the significance. The levels in diabetics are higher than healthy controls and the difference is strongly significant (P<0.01).

Figure 3: Bar diagram depicting Comparision of HbA1C between group I, II, III

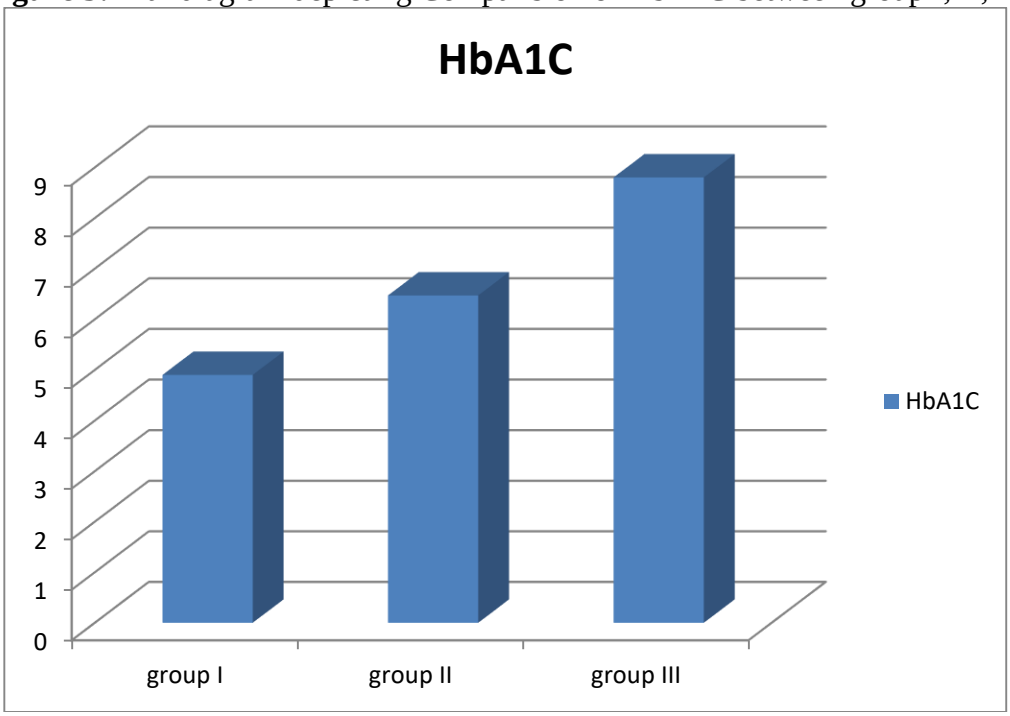
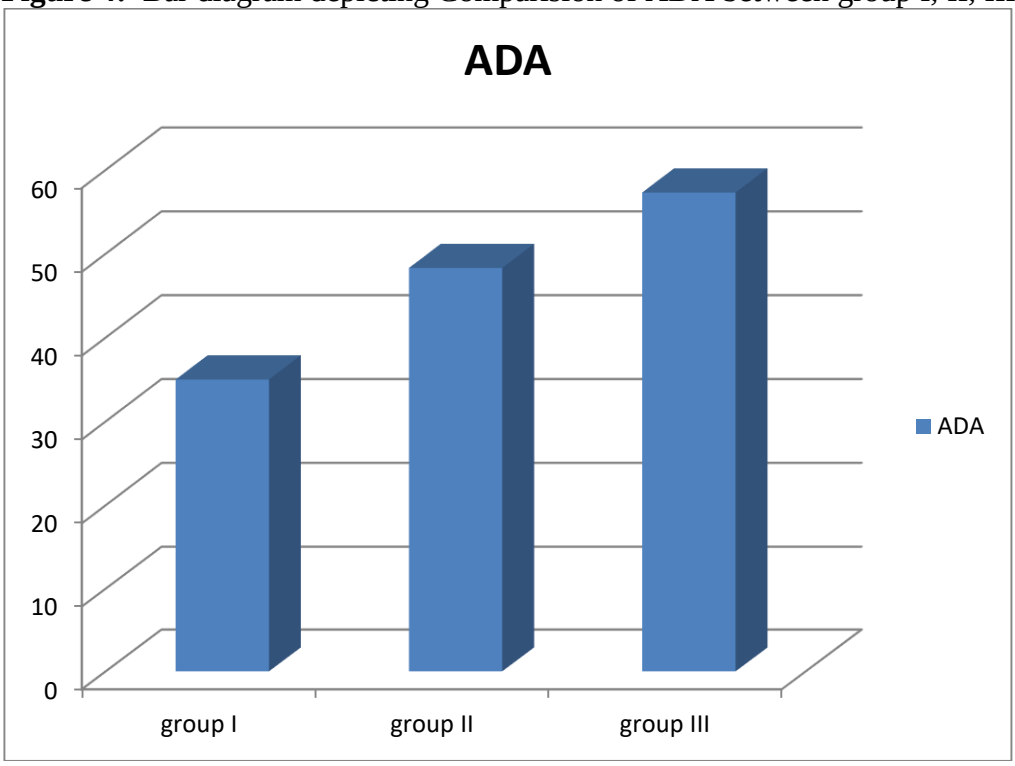


Figure 4: Bar diagram depicting Comparision of ADA between group I, II, III



ADA values in group I, group II, group III are 35 ± 4.5, 48 ± 2.97 and 57.3 ± 3.34 respectively. Student t test (two tailed) has been used to find the significance. The levels in diabetics are higher than healthy controls and the difference is strongly significant (P<0.05).

Figure 5: Scatter plot showing correlation between serum ADA levels & HbA1C in control group

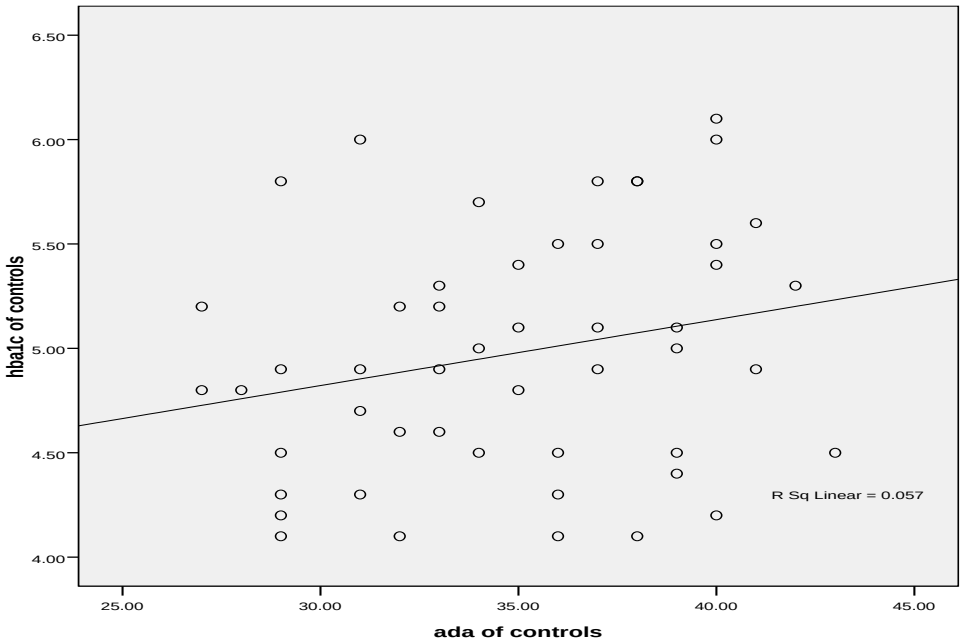
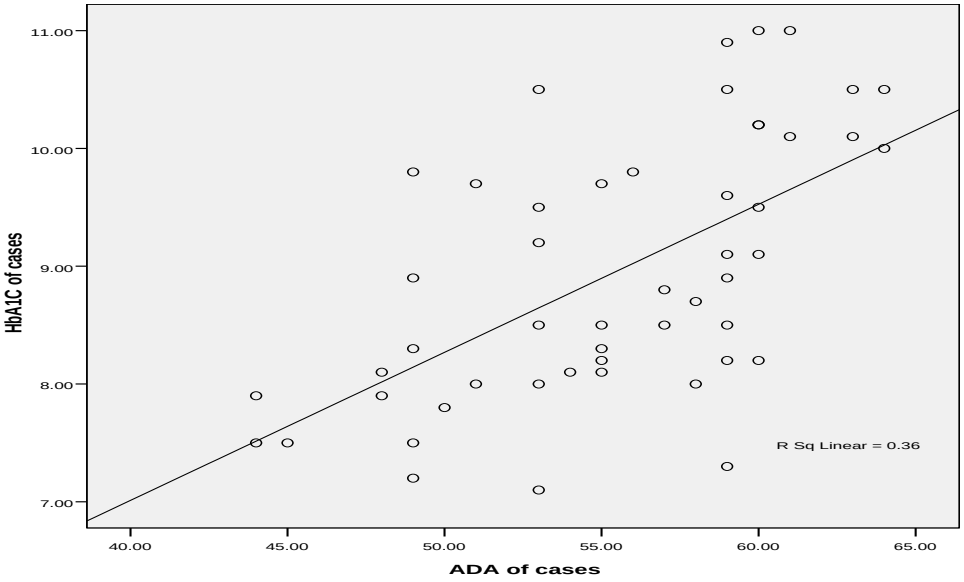


Figure 6: Scatter plot showing correlation between serum ADA levels & HbA1C in diabetic group.



Correlation of ADA levels with HbA1C in all three groups: 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 (HbA_{1C}) were performed.

The blood glucose levels & glycosylated haemoglobin (glycemic marker) are monitored to estimate the glycemic status of the patient.

The fasting plasma sugar was done to assess the short term glycaemic control. The FPS values in

control Group Iwas 90.8 ±10.23, Study Group II was 112 ±4.92 and Study Group III was 142.06 ± 12.5. The difference in short term glycaemic control (FPS) values between the two study groups was statistically significant (P<0.02).
PPBS values in control Group I was 140 ±7.26, Study Group II was 148 ± 6.25 and Study Group III was 192.6 ± 10.9. The levels among diabetics are higher than healthy controls and the difference is strongly significant (P<0.04).

To assess the long term glycaemic control HbA_{1C} levels were measured. Mean HbA_{1C} values in control Group I (4.96 ± 0.6), Study Group II (6.47± 0.34) and Study Group III (8.86 ± 1.2). Student t test (two tailed) has been used to find the significance. The levels in diabetics are higher than healthy controls and the difference is strongly significant (P<0.01).
ADA values in group I, group II, group III are 35 ± 4.5, 48 ± 2.97 and 57.3 ± 3.34 I/L respectively. Student t test (two tailed) has been used to find the significance. The levels in diabetics are higher than healthy controls and the difference is statistically significant (P<0.05).

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 HbA_{1C} levels.

Adenosine deaminase (ADA) acts on adenosine and several other adenosine nucleosideanalogues. 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.

Mechanism of ADA causing insulin resistance: 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 mellitus patients. Kurtul N et al[12] have shown increased level of serum ADA activity in type 2 DM patients and its correlation with HbA1c and suggested that ADA is an important enzyme for modulating the bioactivity of insulin. 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[13] 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). 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. [15] 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. Therefore, ADA may serve as an immunoenzyme marker in the aetiopathology of

type 2 DM[11]. Anjali C. Warrier et al has shown increased ADA activity and its correlation with hyperglycemia (glycated hemoglobin) and lipid peroxidation in DM patients. [16] 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[8] 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 was 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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