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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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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

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 could be due to altered insulin related T-lymphocyte function. Hyperglycaemia is associated with increased level of (ADA), which is one of the factor which leads to increase oxidative stress level by generatingthe reactve oxygen species (ROS) leading to insulin resistance. In our study, ADA level was significantly high in controlled diabetes mellitus type 2 (group II with HbA1c < 7) and was much higher in uncontrolled diabetics (group III with HbA1c > 7) compared to healthy controls (group I). The present study was aimed to find the level of Serum (ADA) among the patients with type 2 diabetes mellitus through a case control study and correlation of Adenosine deaminase with glycemic control (HbA1c). Comparison of the parameters fasting plasma glucose (FPG), post prandial plasma glucose (PPPG) HbA1c, and ADA between the 3 groups were done using Student t test and was statistically significant. Pearson's coefficient correlation was done between ADA and HbA1c and a positive correlaion was seen that was also statistically significant.

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This indicates that ADA ses 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.

Keywords: Diabetes mellitus; adenosine deaminase; advanced glycation end products; HbA1c.

1. INTRODUCTION

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. It consistently demonstrates three cardinal abnormalities namely, resistance to the action of insulin in peripheral tissues particularly muscle and adipose tissue, decreased insulin secretion and 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. 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 to be a marker for insulin function [12,13]. But its connection with the immune system was not yet established in the patients with diabetes mellitus type 2. Even though there are some reports available on adenosine deaminase levels in the patients with diabetes mellitus type 2, 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 (what do you mean?) and understand its importance in the immunopathogenesis of type 2 diabetes mellitus.

2. MATERIALS AND METHODS

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

2.1 Study Individuals were divided into 3 Groups

- Group I comprised of 40 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 HbA1c<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. Age, gender, height, weight, DM duration, general history and medications were recorded from the participants. Blood

samples were collected following overnight fasting.

2.2 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 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).

3. RESULTS AND DISCUSSION

The study population comprised of a total of 100 individuals, of which 60 were Diabetic and 40 were healthy controls. All the study individuals were in the age group between 30 and 60 years and individuals in control group were age and sex matched with diabetic cases.

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.

Table 1. Descriptive statistics for group I

	AGE	SEX	FBS	PPBS	HbA1c	ADA
N Valid	40	40	40	40	40	40
Mean	49.2750		90.8250	140.0000	4.9675	35.0000
Std. Deviation	8.95570		10.23791	7.26071	0.60272	4.50071

Table 2. Descriptive statistics for group II

	AGE	SEX	FBS	PPBS	HbA1c	ADA
N Valid	30	30	30	30	30	30
Mean	52.0667		112.0000	148.9333	6.4767	48.3000
Std. Deviation	9.13098		4.92705	6.25842	0.34209	2.97287

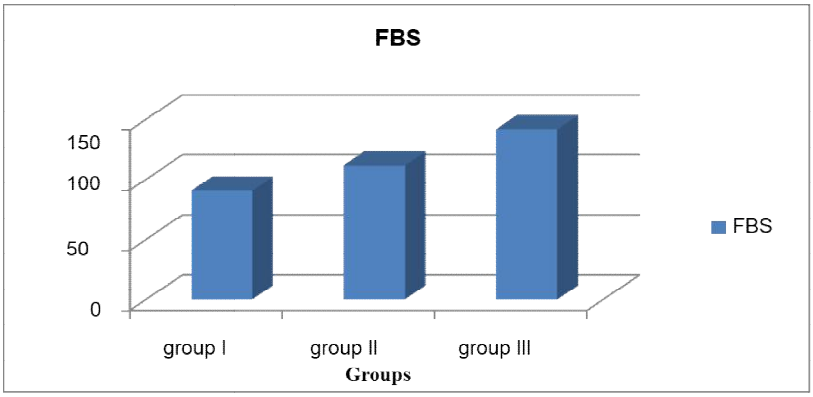

Fig. 1. Bar diagram depicting Comparison of FBS between group I, II ,III

Table 3. Descriptive statistics for group III

	AGE	SEX	FBS	PPBS	HbA1c	ADA
N Valid	30	30	30	30	30	30
Mean	47.2667		142.0667	192.6000	8.8633	57.3000
Std. Deviation	8.40744		12.58607	10.99404	1.20129	3.34406

*All the biochemical study parameters were analysed using Statistical Package for the Social Sciences Statistical tests used were Descriptives, Student t test & Pearson's Correlation. *P < 0.05 is significant. **P < 0.001 is strongly significant.*

Table 4. A. Comparison of FBS between group I & II Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
FBS I & II	-0.084	28	0.934	-0.35294	4.20193	- 8.96020	8.25431

Table 4 – B. Comparison of FBS between group II & III: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
?FBS III	-0.321	28	0.751	- 1.51131	4.71062	- 11.16058	8.13795

Table 4 –C. Comparison of FBS between group I & III: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
FBS I & III	2.32	28	0.028	10.02715	4.32211	1.17371	18.88059

Table 4 & Fig. 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).

Table 5 & Fig. 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).

Table 6 & Fig. 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).

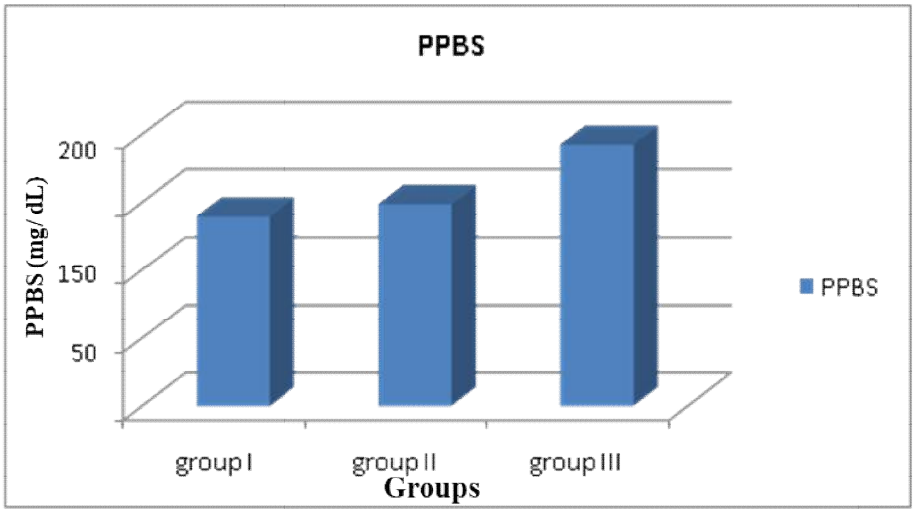

Fig. 2. Bar diagram depicting Comparison of PPBS between group I, II ,III

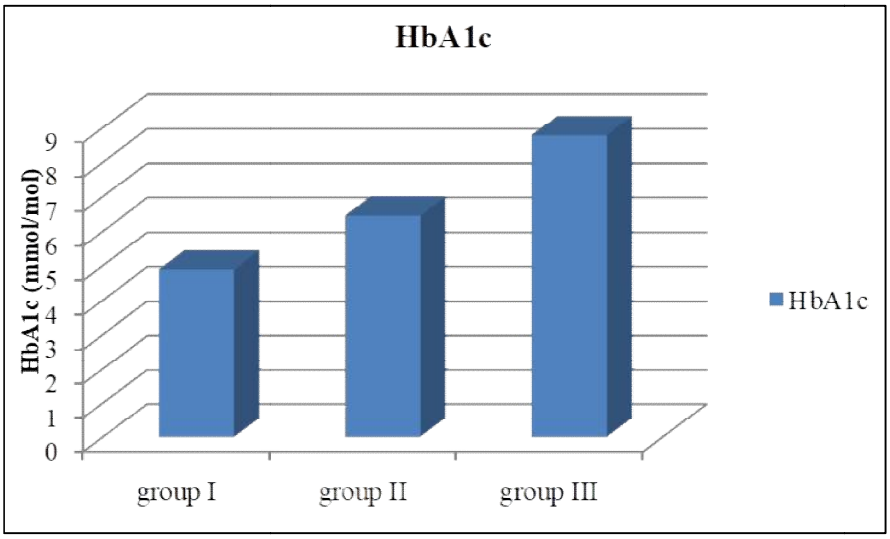

Fig. 3. Bar diagram depicting Comparison of HbA1c between group I, II, III

Table 5 – A. Comparison of PPBS between group I & II: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
PPBSI	-.032	28	.974	-.08036	2.48848	- 5.17778	5.01706

Table 5 – B. Comparison of PPBS between group II & III: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
PPBS3	-.347	28	.731	- 1.41964	4.08582	- 9.78908	6.94979

Table 5 – C. Comparison of PPBS between group I & III: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
PPBS3	.850	28	.42	3.50000	4.11697	- 4.93322	11.93322

Table 6 – A. Comparison of HbA1c between group I & II: Independent Samples Test

	t- test for Equality of Means						
	T value	**Degrees of freedom (df)**	**Significance. (2-tailed)**	**Mean Difference**	**Standard. Error (SE) Difference**	**95% Confidence Interval of the Difference**	
HbA1c HbA1c	.249	28	0.805	0.06111	0.24562	-0.44203	0.56425

Table 6 – B. Comparison of HbA1c between group II & III: Independent Samples Test

	t- test for Equality of Means						
	T value	Degrees of freedom (df)	Significance. (2-tailed)	Mean Difference	Standard. Error (SE) Difference	95% Confidence Interval of the Difference	
HbA1cHbA1c	-.564	28	0.577	-0.25556	0.45305	- 1.18359	0.67248

Table 6 – C. Comparison of HbA1c between group I & III: Independent Samples Test

	t- test for Equality of Means						
	T value	Degrees of freedom (df)	Significance. (2-tailed)	Mean Difference	Standard. Error (SE) Difference	95% Confidence Interval of the Difference	
HbA1c HbA1c	1.728	28	0.095	0.73482	0.42531	-0.13639	1.60603

Table 7 – A. Comparison of ADA between group I & II: Independent Samples Test

	t- test for Equality of Means						
	T value	Degrees of freedom (df)	Significance. (2-tailed)	Mean Difference	Standard. Error (SE) Difference	95% Confidence Interval of the Difference	
ada1	-.445	28	0.660	-0.77512	1.74063	- 4.34065	2.79041

Table 7 – B. Comparison of ADA between group II & III: Independent Samples Test

	t- test for Equality of Means						
	T value	Degrees of freedom (df)	Significance. (2-tailed)	Mean Difference	Standard. Error (SE) Difference	95% Confidence Interval of the Difference	
ada3	- 1.467	28	0.153	- 1.82297	1.24251	- 4.36813	0.72219

Table 7 – C. Comparison of ADA between group I & III: Independent Samples Test

	t- test for Equality of Means						
	T value	Degrees of freedom (df)	Significance. (2-tailed)	Mean Difference	Standard. Error (SE) Difference	95% Confidence Interval of the Difference	
ada3	1.309	28	0.235	1.61111	1.23123	-.91095	4.13317

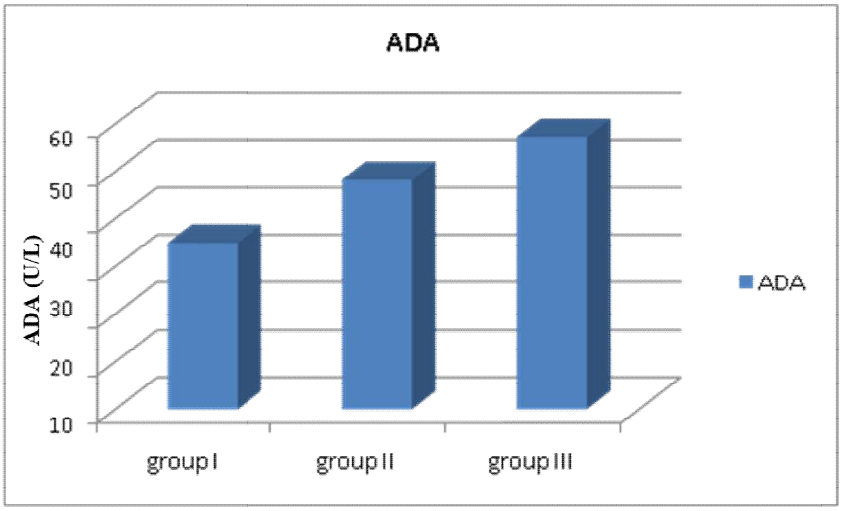

Fig. 4. Bar diagram depicting Comparison of ADA between group I, II ,III

Table 7 & Fig. 4. 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).

Correlation of ADA levels with HbA1c in all three groups.

Table 8 – A. Correlation of ADA and HbA1c in group I (healthy controls)

		ADA	HbA1c
ADA	Pearson Correlation	1	0.297
	Sig. (2- tailed)		0.063
	N	40	40
HbA1c	Pearson Correlation	.297	1
	Sig. (2- tailed)	0.063	
	N	40	40

Table 8 – B. Correlation of ADA and HbA1c in group II (diabetics with HbA1c < 7)

		HbA1c	ADA
HbA1c	Pearson Correlation	1	.370(*)
	Sig. (2- tailed)		0.044
	N	30	30
ADA	Pearson Correlation	0.370(*)	1
	Sig. (2- tailed)	0.044	
	N	30	30

** Correlation is significant at the 0.05 level (2-tailed).*

Table 8 – C. Correlation of ADA and HbA1c in group III (diabetics with HbA1c > 7)

		HbA1c	ADA
HbA1c	Pearson Correlation	1	.672(**)
	Sig. (2- tailed)		0.000
	N	30	30
ADA	Pearson Correlation	0.672(**)	1
	Sig. (2- tailed)	0.000	
	N	30	30

*** Correlation is significant at the 0.01 level (2 -tailed).*

Table 8 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).

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 glucose (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. The fasting plasma glucose was done to assess the short term glycaemic control. The FPS values in control Group I was 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.

All these data are shown in the tables above. Do not repeat! HbA1cHbA1cHbA1cAll these data are shown in the tables above. Do not repeat!

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 [15]. Also, ADA is considered to be a marker of T cell activation and a producer of reactive oxygen species (ROS) [16]. 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 [17].

3.1 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. [18]. 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 [19]. Various studies Who? show altered Adenosine deaminase level in type 2 DM. Most of them showed increased adenosine deaminase activity in type 2 diabetes mellitus patients [20-21]. Kurtul N et al (2004)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 [22]. Also suggest that ADA play important role in insulin effect and glycemic control. Increased activity of ADA might be marker for insulin. Hoshino 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). ADA2 activity in the poorly controlled NIDDM patients directly correlated with the HbA1c level.

The significant increase in adenosine deaminase activity in diabetic subjects tend to be found for having altered the immunity in animals [23]. Therefore, ADA may serve as an immunoenzyme marker in the aetiopathology of type 2 DM [11].. Our study data also coincided with the previous literatures and estimates that Adenosine deaminase (ADA) levels was significantly high in type II diabetics than healthy controls .

4. 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.

CONSENT AND ETHICAL APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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