

Original Article

Role of Adenosine Deaminase to Predict Glycemic Status in Type 2 Diabetes Mellitus

Nisha Subhashchandra Ramani, Krishnamurthy N , Raghavendra Prasad B N ¹,

Ashakiran S, Sumathi M.E, Harish R

*Departments of Biochemistry, Medicine'
Sri Devaraj Urs Medical College, Kolar*

ABSTRACT

Introduction: Diabetes mellitus (DM) is a heterogeneous disease characterized by an abnormal or relative deficiency of insulin and insulin resistance. Adenosine Deaminase (ADA) is an enzyme involved in purine metabolism. Literature demonstrates that in the patients with type 2 DM, the level of ADA is higher than that of non-diabetics.

Objectives: 1. To estimate the serum ADA in patients with type 2 DM.

2. To correlate ADA levels with HbA_{1c} (for glycemic status) in patients with type 2 DM.

Methods: The present study is a case control study. It includes 101 subjects, including 51 cases of type 2 diabetes mellitus and 50 age and sex matched healthy controls. Adenosine Deaminase, HbA_{1c}, fasting and postprandial blood glucose levels were measured and the results were compared with controls.

Results: An elevation of serum ADA was found in diabetic subjects as compared to controls. Mean and Standard deviation of serum ADA in cases and controls was 32.06 ± 17.09 and 19.28 ± 5.59 respectively. Serum ADA is significantly higher in cases as compared to controls ($p < 0.001$). ADA activity is correlated with fasting blood glucose ($r = 0.694$, $p < 0.001$), postprandial blood glucose ($r = 0.652$, $p < 0.001$) and HbA_{1c} ($r = 0.290$, $p < 0.05$). Correlation is positive and significant.

Conclusion: Serum ADA is elevated in the patients with diabetes as compared to controls. This reflects that serum ADA can be considered as a marker of glycemic status in the diabetic population.

Key words: Adenosine, Adenosine Deaminase, Blood glucose, HbA_{1c}, Type 2 diabetes mellitus.

Corresponding author:

Dr. Krishnamurthy N

Professor and HOD,

Department of Biochemistry,

Sri Devaraj Urs Medical College,

Kolar-563101

Karnataka,

India.

Mobile: 09886848234

Email ID: krisvita@rediffmail.com

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from abnormal or relative deficiency of insulin and insulin resistance. The latest WHO Global Burden of Disease estimates the worldwide burden of diabetes in adults to be around 173 million in the year 2002. Around two thirds of these live in developing countries.

Diabetes is no longer a condition of developed, 'industrialized' or 'Western' countries.^[1]

Type 2 diabetes is characterised by insulin resistance where there is impaired ability of the hormone to suppress hepatic glucose output and to promote peripheral glucose disposal and compromised function of pancreatic β cells such that insulin secretion is insufficient to match the degree of insulin resistance.^[2]

The prevalence of obesity and type 2 DM is increasing rapidly. Obesity and type 2 diabetes are associated with metabolic alterations, such as elevated plasma fatty acids and a reduced ability of insulin to suppress lipolysis, which lead to the accumulation of intramyocellular lipid. This accumulation of lipid within muscle cells has been linked to the development of insulin resistance.^[2]

ADA (Adenosine Deaminase) is an enzyme involved in purine metabolism. ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction.^[3,4]

Literature suggests that the levels of adenosine are reduced by ADA. Adenosine is called as a retaliatory metabolite. It has an anti-lipolytic property and through this effect it reduces free fatty acid level. Adenosine Deaminase increases basal and noradrenaline stimulated lipolysis in adipocytes.^[5-7]

Studies have shown that in the patients with type 2 DM, the level of ADA is higher than that of non-diabetics.^[3,8,9,10] In patients with type 2 diabetes, insulin administration has been shown to reduce the elevated ADA levels.^[11]

The half life of serum ADA is about 30 minutes.^[12] Adenosine metabolism also exhibit significant diurnal variations in human blood. In

humans adenosine nucleotides undergo important changes during the dark period, mainly decrease in ATP and enhancement of ADP and AMP levels. This observation might represent a local metabolic adjustment in the energy status of blood cells. Blood adenosine deaminase shows a major peak at 08:00 hr, with almost no changes throughout the rest of the 24 hr. Adenosine-metabolizing enzymes functions as a chemical messenger or a metabolic modulator. Thus there are temporal variations in metabolites and enzymatic activities related to adenosine metabolism in the blood of human volunteers.^[13]

In addition to its association with diabetes, serum ADA activity is also increased in patients with liver cirrhosis as well as in patient with infectious diseases such as hepatitis, tuberculosis, brucellosis, and typhoid fever. The adipocytes produce large amounts of inflammatory cytokines than normal. Immune cells are already present in the close proximity of adipocytes and macrophages easily infiltrate the adipose tissues. This inflammation is also associated with insulin resistance. Adenosine is an endogenous regulator of many different functions of immune system. Adenosine receptors can also be drug targets in adipose tissue to suppress the underlying inflammation in obesity and increase insulin sensitivity.^[3,5]

The concentration of intracellular and extracellular adenosine is regulated by Adenosine Deaminase. There has been increase in the expression of ADA in the conditions like hypoxia which lead to elevated adenosine formation and release.^[14]

Adenosine exerts potent anti-lipolytic effects through the A1 receptor which is the only adenosine receptor expressed in the adipose

tissue. A1 receptor agonists, through its anti lipolytic property decrease free fatty acid levels hence increase insulin sensitivity.^[6]

The elevation of serum ADA in type 2 DM is explained through extracellular cyclic AMP adenosine pathway. The present study aims to estimate the serum ADA in patients with type 2 DM and to correlate ADA levels with HbA_{1c} (for glycemic status) in patients with type 2 DM.

MATERIALS AND METHODS

The present study is a hospital based case control study. The study group and controls are selected from patients and healthy individuals visiting the outpatients /clinical lab and Inpatients of RL Jalappa Hospital and Research Centre, Kolar, India. This study includes 101 subjects of which 51 are cases and 50 are controls.

Inclusion Criteria: Clinically diagnosed cases of type 2 diabetes mellitus are included in the study and this is based on 2010 American Diabetic Association criteria.^[15] Cases are in the age group of 35-75 yrs and are on oral hypoglycemic drugs. Any patient with history of type 2 DM for more than 5 years are included in the study. Age and sex matched physically healthy volunteers with no history of diabetes mellitus or any other chronic diseases are selected as controls. A thorough clinical examination and appropriate investigations were done before selecting the cases and controls for the study.

Exclusion Criteria:

1. Non diabetic cases.
2. Patients with type 2 diabetes mellitus with any other concurrent chronic disease such as Cardiac

diseases, thrombotic stroke, Tuberculosis, Rheumatoid Arthritis, Sarcoidosis, Gout, Renal failure or any other condition which alters the ADA levels in the serum.

3. Gestational diabetes mellitus.

4. Patients with type 2 diabetes mellitus on insulin treatment.

Institutional ethical committee clearance was taken before the start of the study. Informed consent was taken from all the subjects. Relevant investigations (FBS, PPBS and HbA_{1c}) were done before selection of the subjects for the study. BMI was calculated using the formulae weight (kg)/height (m²).^[16] The estimations of ADA were done using the ADA-MTB kit from Micro express, a division of Tulip Diagnostics (P) Ltd by Colorimetric method described by Giusti and Galanti.^[17] Estimation of blood glucose was done by glucose oxidase / peroxidase method.^[18] Estimation of HbA_{1c} was done by weakly binding cation exchange resin method.^[19]

STATISTICAL ANALYSIS

Statistical Analysis was done by student 't' test using SPSS windows version 10.0 software and results were expressed as mean \pm SD. Pearson's bivariate correlation analysis was used to correlate each variable with ADA activity. p values less than 0.05 was considered statistically significant.

RESULTS

As depicted in the figure 1, the mean \pm SD of Age (yrs.) in cases and controls is 49.12 \pm 9.177 and 48.4 \pm 8.822 respectively and they are age matched. As shown in figure 2, the

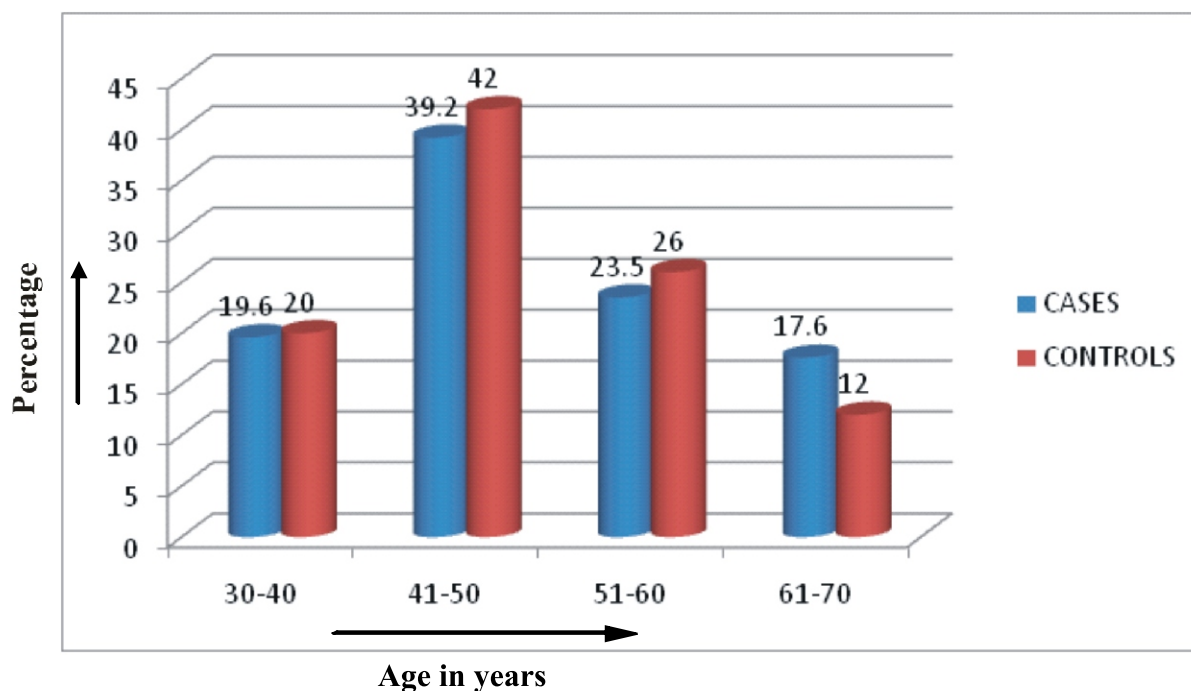


Fig. 1: Age distribution in cases and controls

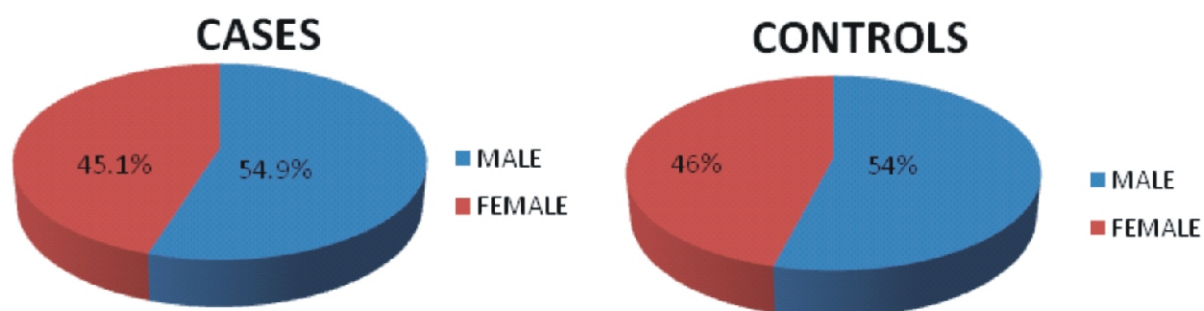


Fig. 2: Gender distribution in cases and controls

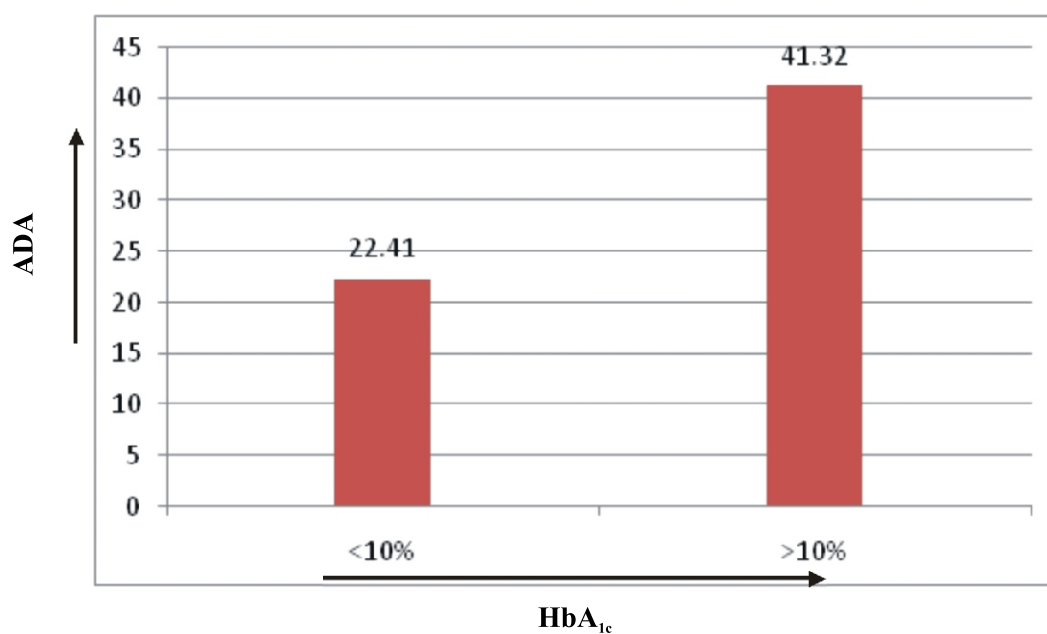


Fig. 3: Comparison of ADA with HbA_{1c}

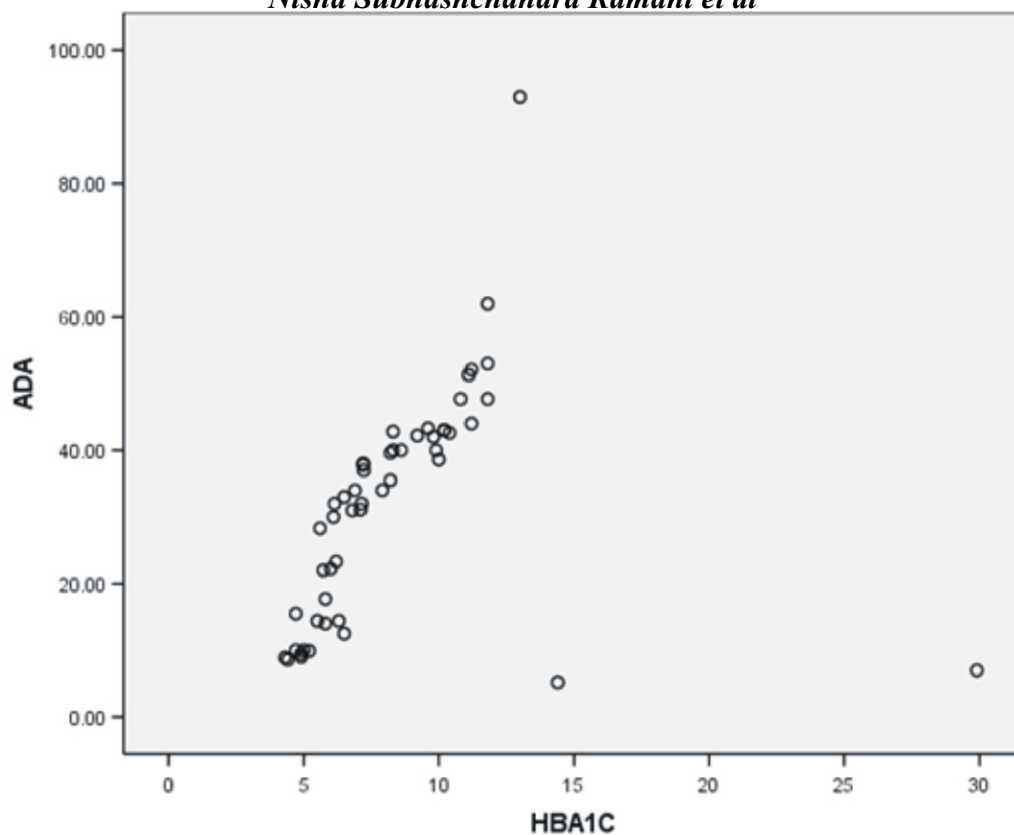


Fig. 4: Pearson's correlation of ADA with HbA_{1c} in study group

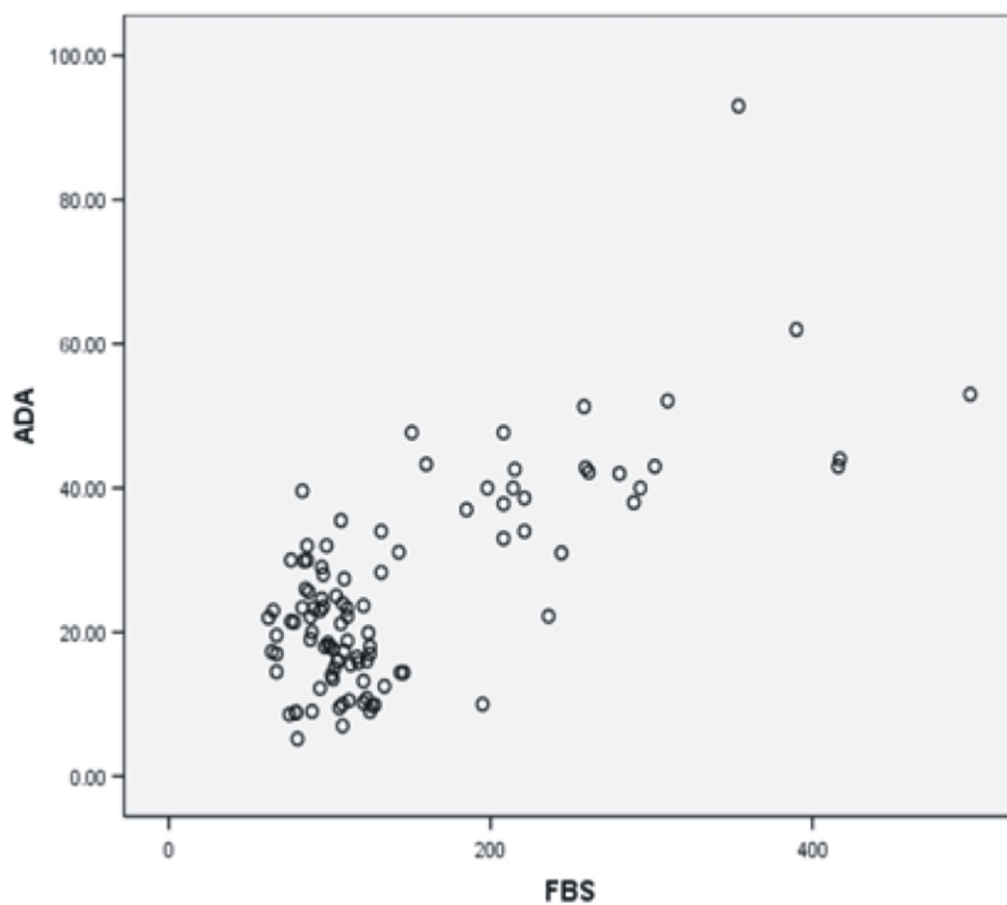


Fig. 5: Pearson's correlation of blood glucose parameters with ADA in study group

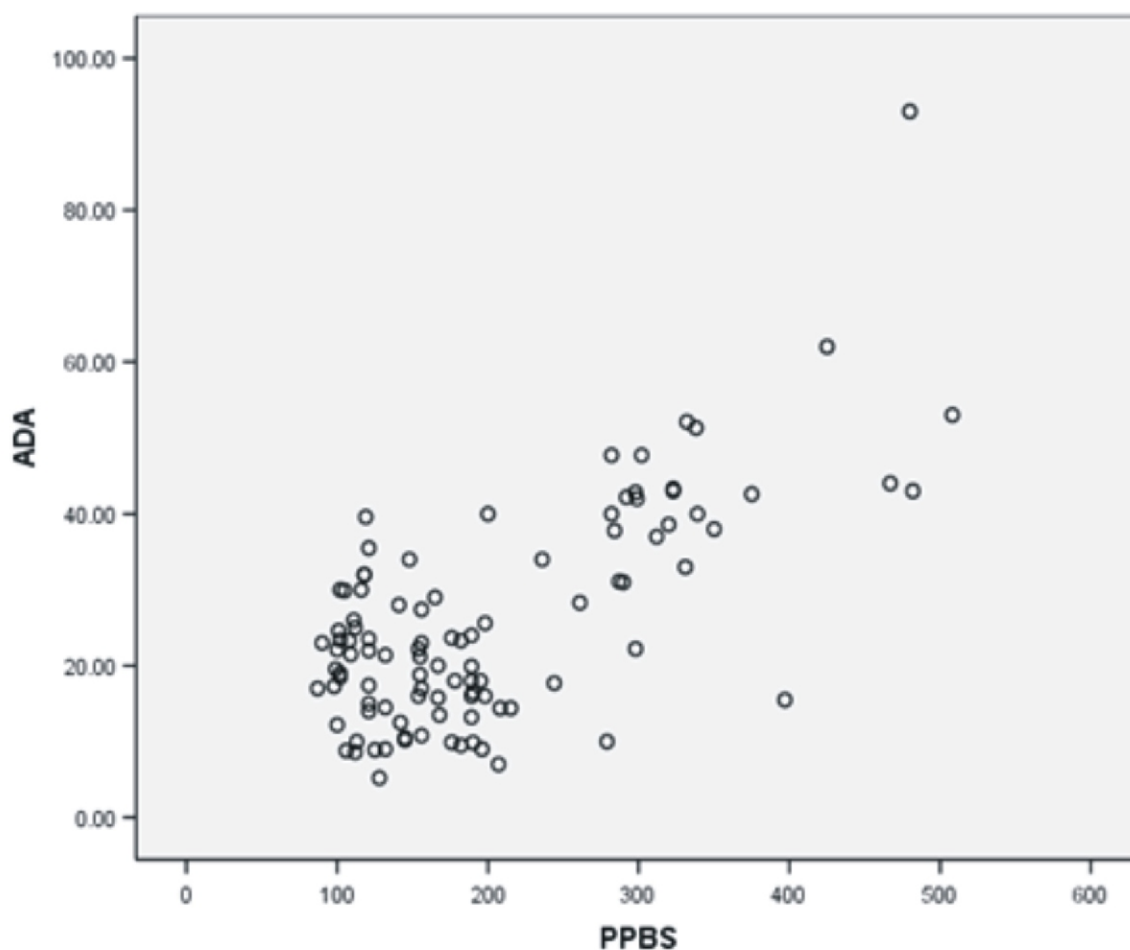


Fig. 5: Pearson's correlation of blood glucose parameters with ADA in study group

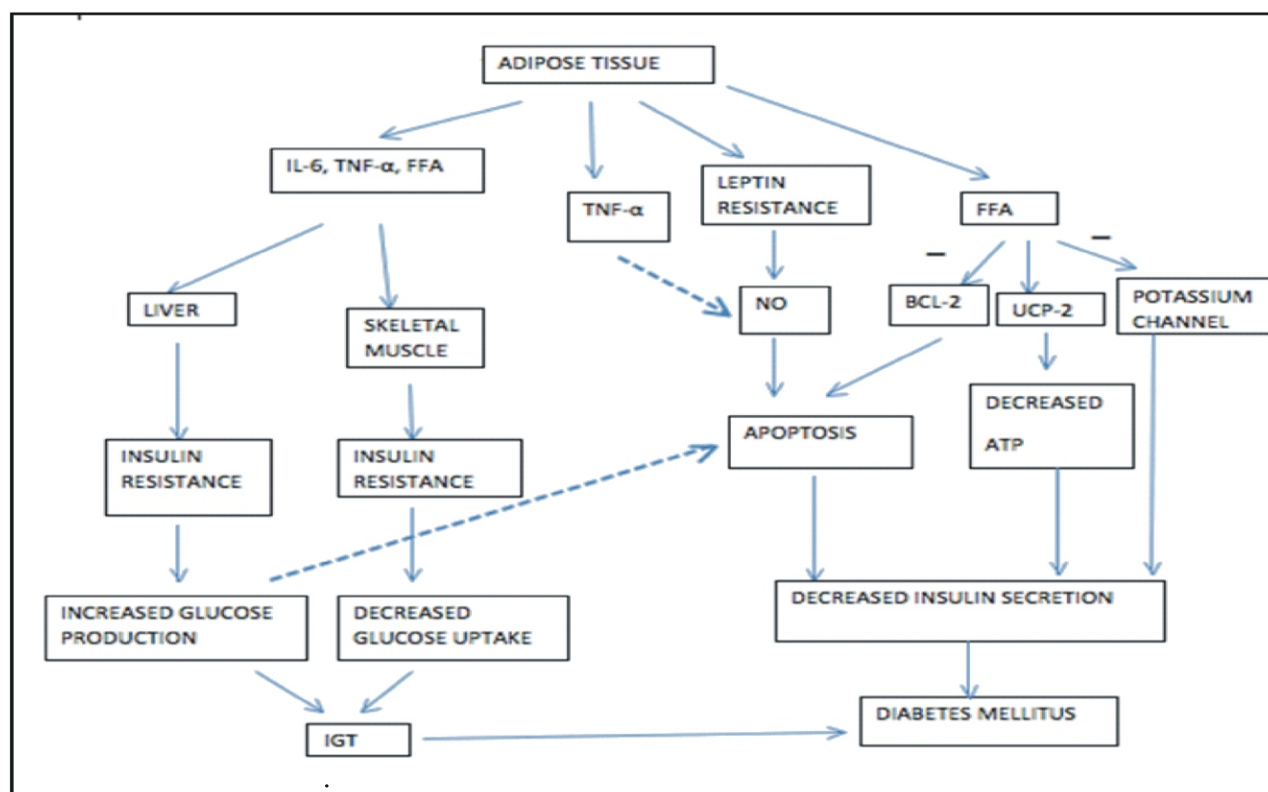


Fig : 6 Adipocyte dysfunction in diabetes and obesity^[27]

Table 1: Mean and SD of FBS, PPBS, HbA1c, ADA and BMI in cases and controls

Parameters	Controls	Cases	'p' value
	Mean \pm SD	Mean \pm SD	
FBS (mg/dl)	98.36 \pm 17.90	192.92 \pm 102.75	<0.001
PPBS (mg/dl)	142 \pm 35.16	261.24 \pm 109.05	<0.001
HbA _{1c} (%)	5.24 \pm 0.62	8.38 \pm 4.00	<0.001
ADA (U/L)	19.28 \pm 5.59	32.06 \pm 17.09	<0.001
BMI	23.27 \pm 4.26	24.62 \pm 3.25	0.082

'p' value of less than 0.05 is significant

cases and controls are gender matched. Among the cases 45.1% are females and 54.9% are males and among the controls 46% are females and 54% are males. Table 1 shows mean and SD of FBS is 192.92 \pm 102.75(cases) and 98.36 \pm 17.90 (controls), of PPBS is 261.24 \pm 109.05(cases) and 142 \pm 35.16(controls), of HbA_{1c} is 8.38 \pm 4.00(cases) and 5.24 \pm 0.62 (controls), of ADA is 32.06 \pm 17.09(cases) and 19.28 \pm 5.59 (controls) and of BMI is 24.62 \pm 3.25(cases) and 23.27 \pm 4.26 (controls)respectively. FBS, PPBS, HbA_{1c} and ADA is significantly higher in cases as compared to controls (p<0.001). As depicted in figure 3, patients with type 2 DM are divided into two groups, those with HbA_{1c}<10 % and those with HbA_{1c}>10%. For HbA_{1c} <10%, the Mean and SD of ADA is 22.41 \pm 9.7 and for HbA_{1c} >10%, the Mean and SD of ADA is 41.32 \pm 16.74 with p < 0.05. Figure 4 shows that ADA activity was correlated with HbA1c (r = 0.290, p <0.05) and the correlation is positive and significant. Figure 5 shows that ADA activity was correlated with FBS (r = 0.694, p < 0.001) and PPBS (r = 0.652, p < 0.001) and a large positive

correlation is found between blood glucose values and ADA and it is significant.

DISCUSSION

Reports from the previous studies done by Reddy M et al showed that as compared to the control group, ADA activity in type 2DM patients was higher.^[8] When glycemic control was relatively good, ADA activity was low. In line with previous reports done by other researchers, ADA activity in type 2DM patients in the present study was significantly higher than that in the control group(mean \pm SD of 32.06 \pm 17.09 vs. 19.28 \pm 5.59, p < 0.001) as depicted in table 1. Moreover, compared to that of type 2 DM patients with relatively good glycemic control (HbA1c< 10%), the ADA activity in type 2DM patients with poorglycemic control (HbA1c> 10%) was significantly lower. These results are depicted in the figure 3 and they are consistent with those reported by Lee JG et al.^[3] Previously, in a study conducted by Shivaprakash M et al on a group of thirty-six adult patients of either sex who had history of not

less than six years of diabetes mellitus and equal number of healthy non-diabetics as controls, showed a significant ($p < 0.001$) increase in adenosine deaminase activity with a mean \pm SD of 37.2 ± 9.29 U/l in diabetic subjects when compared to controls who had normal mean \pm SD values of 18.2 ± 5.6 U/l.^[9]

As shown in figure 5, a large positive and significant correlation exists between the blood glucose levels and serum ADA level with $r = 0.6$ and $p < 0.001$. A positive correlation is also found between long term index of glycemic control i.e. glycated hemoglobin (HbA1c) and serum ADA as depicted in figure 4. This finding is in agreement with some of the previous studies which show significant correlation between HbA1c and ADA levels.^[3,20,21,22]

In the analysis of all patients, ADA activity did not have a significant correlation with age, gender or duration of diabetes.^[23,24]

In addition, in a study conducted by Pawelczyk T et al found that the serum ADA levels return to normal following insulin infusion.^[11] However in the present study such intervention of the effect of insulin treatment on serum ADA levels could not be tested because of the inclusion criteria as defined in the selection of subjects.

As shown in figure 6, excess postprandial lipids and glucose circulate through the blood stream and are taken up by the pancreas, the liver, and adipose tissue. The adipocyte stores triglycerides in the lipid droplet, leading to adipocyte hypertrophy. These exposures in excess lead to cellular dysfunction increased circulating free fatty acids and a proinflammatory state. Exposing the

hepatocytes to excess fats and carbohydrates leads to steatohepatitis and insulin resistance. Thus, there is elevation of free fatty acid levels in diabetes due to increased lipolysis which leads to worsening insulin resistance and beta cell failure.^[25,26,27]

Adenosine is an endogenous purine nucleotide that modulates many physiological processes. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A1, A2A, A2B, and A3).^[6,28]

Endogenous adenosine is supposed to be an important regulator of adipose tissue metabolism by increasing the sensitivity of adipocyte glucose transport and oxidation and by inhibiting lipolysis potently. Pharmacological studies have suggested that the antilipolytic effect of adenosine is mediated via the A1 receptor. In addition, it has also been shown that the basal levels of endogenous adenosine are sufficient to cause inhibition of lipolysis. Stimulation of inhibitory Gi protein-coupled receptors leads to inhibition of adenylyl cyclase and decreased

cAMP levels and lipolysis. Increased lipolysis is associated with increased levels of cyclic AMP (cAMP).^[6,29]

It has been shown that in the adipose tissue most of the adenosine is formed extracellularly. Extracellular adenosine is metabolized by adenosine deaminase. Thus, increased formation of adenosine leads to increased adenosine deaminase. The source of extracellular formation of adenosine is via an enzymatic cascade for the breakdown of ATP, ADP and AMP which appears to be the major mechanism that leads to elevated extracellular

adenosine. Also there is accumulating evidence that cAMP, a well-known intracellular second messenger may also be adenosine precursor. Extracellular conversion of cAMP to adenosine is achieved by sequential activities of ectophosphodiesterase and CD73. Thus CD73 appears to be an important point of convergence for the extracellular formation of adenosine derived from ATP or cAMP. The concept of extracellular cAMP-adenosine pathway has been studied in many cell types but was explored in most detail in kidney. The effect of extracellular conversion of cAMP to adenosine would there by depend on the type of adenosine receptors expressed on the target and neighboring cells, where A1/A3 receptors attenuate cAMP levels and A2A and A2B receptors would increase cAMP levels.^[30,31]

The presence of extra cellular cAMP adenosine pathway is suggested by three findings. First cAMP exists in isolated adipocytes in response to beta adrenoceptor and adenylatecyclase stimulation. Second cAMP is metabolized to AMP and adenosine in adipocyte plasma membranes by ecto-phosphodiesterase and 5'nucleotidaserespectively. Third, cAMP and adenosine appear in the extracellular fluid of adiposetissue. Studies have shown, when increasing doses of cAMP were perfused into adiposetissue through micro dialysis, corresponding levels of AMP and adenosine increased.^[32,33]

Adenosine exerts its protective effects by inhibiting lipolysis through A1 receptors. Adenosine deaminase inactivates adenosine and hence activates lipolysis and markedly potentiates the increase in cAMP accumulation

due to norepinephrine.^[6, 28] As discussed in the pathophysiology of diabetes, deregulated fat metabolism and consequent elevation of free fatty acids leads to subsequent development of type 2 Diabetes Mellitus.^[26, 27]

CONCLUSION

A case-control study using age and sex matched controls is used to evaluate serum Adenosine Deaminase (ADA) levels in patients with type 2 DM. Serum levels of ADA were found to be significantly higher in type 2DM when compared to controls. ADA activity was positively correlated with HBA1c and blood glucose values. Age, gender and duration of diabetes were not found to significantly influence the ADA level.

Though there is a clear cut elevation of serum ADA in type 2 diabetes mellitus, due to short half life and diurnal variations of ADA, the role of serum ADA as a marker of glycemic status in patients with type 2 diabetes mellitus need further studies.

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