

**Original Research Article****Role of Adenosine Deaminase and Insulin in Type 2 Diabetes Mellitus in Eastern India**

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Email: [vineetkhemka95@gmail.com](mailto:vineetkhemka95@gmail.com), Ph: +91- 9903290420 (M)**ABSTRACT****Background & Objectives:** Adenosine plays a crucial role in bioactivity of insulin and regulates insulin activity in type 2 diabetes mellitus (T2DM). The aim of our study was to study the altered levels of adenosine deaminase and its association with insulin in T2DM.**Methods:** The preliminary case-control study included age, sex and body mass index (BMI) matched 68 T2DM cases and 52 healthy control subjects. Serum ADA was measured using a spectrophotometer while fasting serum levels of insulin was measured by commercially available immunoassay kits and routine biochemical parameters were analyzed in all study groups.**Results:** Serum ADA level was found significantly higher among T2DM subjects with respect to controls ( $38.77 \pm 14.29$  versus  $17.02 \pm 5.74$  U/L;  $P < 0.0001$ ). An increase in serum insulin levels were also observed in T2DM cases as compared to controls and were statistically significant ( $17.28 \pm 6.71$  versus  $10.33 \pm 3.89$  U/L;  $P < 0.0001$ ). Serum ADA level showed a significant positive correlation with fasting plasma glucose ( $r = 0.324$ ;  $P = 0.007$ ) level among non-obese T2DM subjects, but no significant correlation was observed in controls ( $r = 0.071$ ;  $P = 0.615$ ).**Conclusion:** The high level of serum ADA suggests that it may be used as a prognostic marker for the pathogenesis of T2DM.**Keywords:** Diabetes Mellitus, Adenosine deaminase, Insulin, Insulin resistance.

## INTRODUCTION

Diabetes Mellitus is one of the most leading endocrinological disorder characterized by chronic hyperglycemia, metabolic abnormalities and long term complications resulting from environmental, genetic and aetiological factors<sup>[1]</sup>. A few related complications are sedentary lifestyle, unhealthy food habits, and the consequent obesity responsible for the T2DM patients worldwide. The prevalence of diabetes is drastically increasing and 300 million people are likely to be effected by 2030 across the globe<sup>[2]</sup>. In India, it is estimated that approx 80 million people would be affected by the disease by 2030<sup>[3]</sup>. Glycosylated haemoglobin which is formed non- enzymatically by a two-step reaction determines the long term control of diabetes mellitus<sup>[4]</sup>. Insulin resistance and impaired insulin secretion are the most important metabolic factors associated with T2DM<sup>[5]</sup>. Immunological disturbance in T2DM contributes to the pathophysiology and has been associated with cell-mediated immunity and inappropriate T-lymphocyte function<sup>[6]</sup>.

Adenosine deaminase, one of the key enzymes of purine metabolism that catalyzes the deamination of adenosine to inosine, is vital for the differentiation and proliferation of T lymphocytes and monocytes, macrophage system, was considered as a suitable marker of cell-mediate immunity<sup>[7]</sup>.

Adenosine plays a crucial role in bioactivity of insulin and regulates insulin activity in various tissues such as liver, myocardium white adipose, and skeletal muscles<sup>[8,9]</sup>. Adenosine modulates the action of insulin in myocardium and adipose tissue and inhibits liver and skeletal muscle. Likewise, it also regulates the action of insulin on glucose and lipid metabolism in adipose tissue<sup>[10]</sup>. Moreover, it has antilipolysis property in adipose tissue and increases insulin sensitivity for glucose transport<sup>[11]</sup>. Adenosine increases 25% of GLUT4 accessibility to cell surface for glucose transportation<sup>[12]</sup>. The measurement of ADA activity in the serum of obese T2DM patients have been carried out and found an increase in ADA activity in those individuals. Adenosine is liable

for increasing glucose uptake into cells<sup>[13]</sup>. Thus, higher ADA activity in insulin sensitive tissue will decrease adenosine levels which in turn decrease glucose uptake into cells. Adenosine has also been shown to enhance gluconeogenesis and glycogenolysis by increasing cyclic AMP (cAMP) by the action of hepatic adenylatecyclase through adenosine A2a receptor binding in liver. Thus it causes an increase of local insulin resistance and glucose output from the liver<sup>[14]</sup>.

The association between serum insulin and ADA is not clear in T2DM subjects and thus, the aim of our study was to study the altered levels of adenosine deaminase and its association with insulin in T2DM.

## MATERIALS AND METHODS

The case control study comprises of 68 cases and 52 healthy controls which were age, sex and BMI matched. The study was carried out in the Department of General Medicine and Department of Biochemistry, IIMSAR, Haldia, over a period of 8 months. The diabetic subjects were taken from the outpatient department of Endocrinology, IIMSAR, while the control subjects were recruited from the subjects coming to the department for a routine health check-up. A written informed consent from the patient and control was obtained after complete explanation of the study. All the patients and controls were clinically examined and routine biochemical tests were analyzed for all subjects prior to selection. The patients on insulin treatment, hypertension, ischemic heart disease, neurological disorders, renal failure, chronic liver disease, cancer, and immunological disorders were excluded from this study. The study was approved by the institutional ethics committee of the institution which follows Helsinki guidelines.

About 4 ml venous blood samples were obtained from the patients as well as controls after 8–10 hours of fasting. All the routine biochemical parameters were analyzed by automated clinical analyzers. The serum ADA level was measured using a spectrophotometer based on the method by Giusti and Galanti<sup>[15]</sup>. ADA activity is described

as U/L. The serum insulin level was measured using elisa technique.

Statistical analysis of different biochemical parameters was performed by Students't-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed "t"-test and for non-parametric Mann-Whitney U "t" test. To find out the correlation between two variables, Pearson's product moment correlation coefficient was used. A value of  $P < 0.05$  was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA). Statistical analysis for sex distributions was evaluated by chi-square test by using statistical software STATA (version 8, Copyright 1984–2003, Stata Corporation, Texas, USA).

**RESULTS**

The demographic and biochemical profile of the T2DM subjects and healthy controls is presented in Table 1. There was no significant difference in age, sex distribution or BMI in either of the two groups between T2DM and control subjects (Table 1). Fasting plasma glucose, HbA1c and serum cholesterol, and serum triglyceride levels were elevated while serum HDL levels were lower in T2DM subjects compared to healthy controls which were found statistically significant (Table 1).

**Table 1:** Demographic and biochemical profile of the subjects

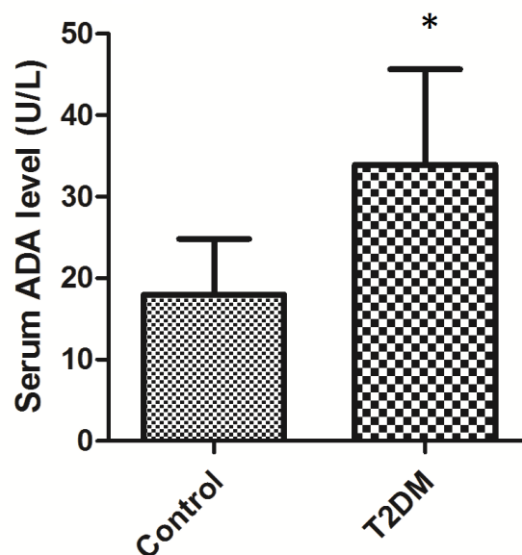
	Control (n = 52)	T2DM (n = 68)
Age(in years)	51.68 ± 6.1	52.09 ± 6.9
Sex (M/F)	30/22	44/24
BMI (kg/m <sup>2</sup> )	25.72 ± 1.64	25.46 ± 1.92
FPG (mg/dl)	82.23 ± 8.52	138.7 ± 31.17*
HOMA IR	1.39 ± 1.24	5.72 ± 2.44*
HbA1c	4.52 ± 0.48	7.92 ± 0.84*
Serum total CHL (mg/dl)	156.3 ± 23.44	188.9 ± 42.3*
Serum HDL (mg/dl)	44.98 ± 6.12	35.22 ± 4.66*
Serum TG (mg/dl)	110.7 ± 19.34	192.8 ± 79.2*

[FPG, fasting plasma glucose; CHL, cholesterol; TG, triacylglyceride; HDL, high density lipoprotein cholesterol. Age, BMI, and serum levels of biochemical parameters were

expressed as the means ± SD. Statistically significant, \*  $p < 0.001$  vs Control.]

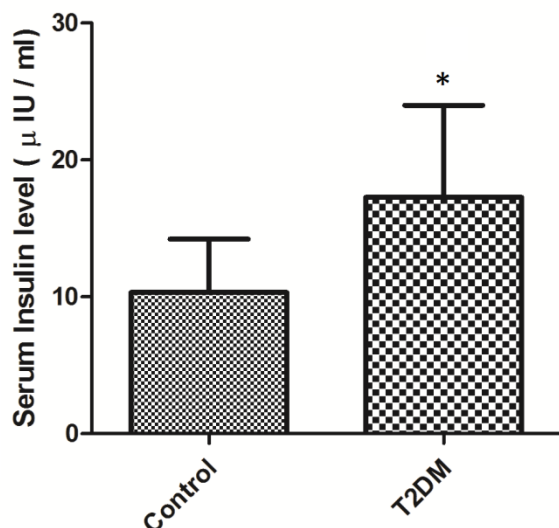
Serum ADA level was found significantly higher among T2DM subjects with respect to controls ( $33.93 \pm 11.74$  versus  $17.96 \pm 6.83$  U/L;  $P < 0.0001$ ) (Figure 1). Moreover, serum insulin levels were also increased in T2DM cases as compared to controls and were statistically significant ( $17.28 \pm 6.71$  versus  $10.33 \pm 3.89$  U/L;  $P < 0.0001$ ) (Figure 2). As presented in Figure 3, serum ADA level showed a significant positive correlation with fasting insulin ( $r = 0.324$ ;  $P = 0.007$ ) level among T2DM subjects, but no significant correlation was observed in controls ( $r = 0.071$ ;  $P = 0.615$ ).

**Fig. 1**

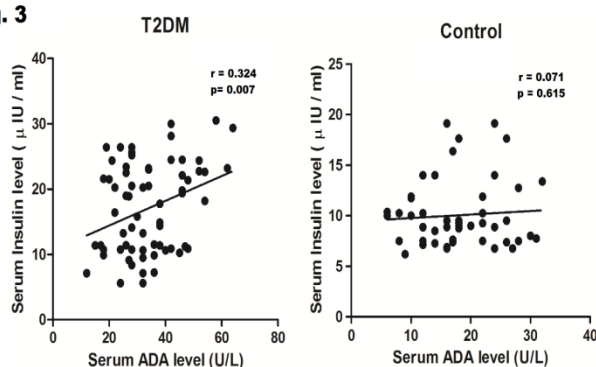


**Fig.1** Serum levels of ADA in control and T2DM subjects.

Serum levels of ADA was determined as described in methods for control and T2DM subjects. Values expressed as the means ± SD. Statistically significant, \*  $p < 0.0001$ , vs T2DM

**Fig. 2****Fig. 2** Serum levels of Insulin in control and T2DM subjects.

Serum levels of Insulin was determined as described in methods for control and T2DM subjects. Values expressed as the means  $\pm$  SD. Statistically significant, \*  $p < 0.0001$ , vs T2DM

**Fig. 3****Fig. 3** XY scatter plots between serum levels of ADA and insulin (A, B) in Control and T2DM subjects. Correlation coefficient (r) represents the degree and nature of correlation between the serum parameters of adiponectin and insulin in controls and T2DM patients as described in the Materials and Methods. A value of  $p < 0.05$  was considered as statistically significant.

## DISCUSSION

Type 2 diabetes is a combination of clinical and biochemical disorders which includes center obesity, hypertension, atherosclerosis, hypertri-

lyceridemia, increased cholesterol and LDL, and decreased HDL. Early identification of insulin resistance helps in minimizing the associated complications. The distribution of ADA varies in different tissues and its highest concentration is found in lymphoid and fatty tissues [16]. Adenosine is known to be involved in insulin mediated glucose uptake in skeletal muscle and its higher activity tends to decrease glucose uptake into cells thereby contributing to insulin resistance [17]. In some *in vivo* and *in vitro* studies, adenosine increases gluconeogenesis and glycogenolysis and stimulates glucose formation and its receptors mainly A1 and A2 adenosine receptors modulates myocardial functions [7].

An increase in the serum levels of ADA in T2DM leads to metabolic changes of insulin especially in adipose tissues where it causes an increase in lipolysis, disturbance in antilipolysis activity and augmentation of hyperlipidemia. The high concentration of fatty free acids (FFA) derived from increased lipolysis activity causes oxidative phosphorylation and ATP retention in adipocytes [18]. Moreover, ADA also impairs PKB and PI3P production in insulin postreceptor phase and reduces insulin sensitivity in adipocytes [19]. ADA also reduces the GLUT4 accessibility to cell surface for glucose transporters [20]. Therefore, the requirement of insulin concentration might be more in diabetic adipocyte cells [21].

DPP-4, an important enzyme acts as an immune regulator by interacting with CD3 and a co stimulator for CD4+ T cells regulates glucose homeostasis by hydrolysing integrins. As adenosine causes apoptosis and inhibits differentiation of T lymphocytes by activating P1 adenosine receptors, interface of ADA with DPP-4 may lead to T cell proliferation and increased cytokine production which can interfere with insulin signalling [22–25]. Cytokines stimulate the acute-phase proteins. In the short term, the acute-phase protein has survival values and regulates homeostasis and in long-term produces diseases [26]. Insulin resistance and hyperglycemia increases the effects and cause the secretion of

few proinflammatory cytokines such as IL-6, and TNF- $\alpha$  which are produced from monocytes, macrophage, and adipose tissue [27]. Aging, sedentary lifestyle, dietary intake, smoking, and obesity are important factors in the increase of peripheral cytokines and leads to immunity disturbance in T2DM and also imbalance the effects of ADA activity [28]. Moreover, ADA deficiency is also associated with impaired immune functions. Thus, suppression of ADA activity may help to improve insulin sensitivity and inflammation, cell proliferation, and T-lymphocyte activity, all of which are associated with the pathophysiology of T2DM. Several reports also suggest that ADA modulates the bioactivity of insulin [29].

Few studies have reported an increased serum ADA levels and also correlated with glycemic control in T2DM patients [30,31]. Moreover, higher serum ADA have also been seen in nonobese T2DM cases and correlated with fasting plasma glucose [7]. Further, metformin, also lowers serum ADA levels and found to be in positive correlation with insulin resistance [32]. Moreover, it has been observed that ADA levels are elevated in T2DM compared to controls and are positively correlated with fasting serum glucose, insulin, and HOMA [32]. Our study shows that serum ADA level is higher in T2DM subjects as compared to controls which are in confirmation with other studies. Further, a strong positive correlation was seen between serum ADA level and fasting serum insulin level which could help in the pathogenesis of T2DM subjects.

However, there are few limitations in our study such as non-estimation of serum transaminase level which are known to be related to ADA. Moreover, a correlation study between serum ADA level and oral glucose tolerance test will further enhance the serum level of ADA in T2DM subjects. Prediabetic subjects were also not considered in this study as screening of serum ADA may be an alarming factor in the pathogenesis of T2DM subjects.

Despite these limitations, our study shows higher serum ADA, and serum insulin levels in T2DM patients and further, a strong positive correlation between ADA and serum insulin which suggests an association between ADA and T2DM subjects. Thus serum ADA may be used as a prognostic marker for the pathogenesis of T2DM although a larger study needs to be done to conclude the fact.

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**Conflict of Interest:** None declared.

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