



Serum Levels of Adenosine Deaminase and Insulin in Type 2 Diabetes Mellitus

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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

Aim: To estimate the levels of serum adenosine deaminase and insulin in patients with type 2 diabetes mellitus and healthy subjects and to find the correlation between serum adenosine deaminase and serum insulin in type 2 diabetes mellitus.

Study Design: Case-control study.

Place and Duration of the Study: Department of Biochemistry and Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur between October 2017 to September 2019.

Methodology: 40 cases of type 2 diabetes mellitus were recruited from the Medicine Outpatient Department, RIMS and 40 healthy controls of age and sex-matched were recruited from those who came for a routine health check-up. Serum adenosine deaminase was measured by the calorimetry method and serum insulin was measured by ELISA method. The data were analyzed using statistical tools like Chi-square test, Independent sample t-test, Pearson's Correlation through SPSS 21.0.

Results: Mean serum adenosine deaminase was (38.97±8.853)U/L in cases and (20.05±5.309)U/L in controls and it was statistically significant ($P<0.001$). Mean serum insulin in cases was found to

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be (18.09±5.554) μ IU whereas in controls, it was (9.06±2.509) μ IU which is statistically significant ($p < 0.001$). Serum adenosine deaminase and insulin were found to be positively correlated to each other ($r = 0.956$, $p < 0.001$).

Conclusion: Serum adenosine deaminase and serum insulin were significantly increased in type 2 diabetes mellitus and positively correlated to each other. Serum adenosine deaminase may be used as a prognostic marker for the pathogenesis of type 2 diabetes mellitus.

Keywords: Adenosine deaminase; type 2 diabetes mellitus; insulin; fasting blood sugar.

1. INTRODUCTION

Diabetes Mellitus is one of the leading endocrinological disorders characterized by chronic hyperglycemia, metabolic abnormalities and long term complications resulting from environmental, genetic and, other aetiological factors [1]. According to recent estimates by the International Diabetes Federation (IDF), approximately 285 million people worldwide (6.6%) in the age group of 20–79 years had diabetes in 2010. The figure is expected to reach 438 million people (7.8%) by 2020. In India, the estimated number of diabetics was 50.8 million in 2010 and expected to rise to 87.0 million by 2030 [2]. Currently, more than 400 million people suffer from diabetes worldwide of which type 2 diabetes mellitus (T2DM) makes up about 90% [3]. The pathophysiology of T2DM is characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and declining β -cell function eventually leading to β -cell destruction [4]. Immunological disturbance in T2DM contributes to the pathophysiology and has been associated with the cell-mediated immunity and inappropriate T-lymphocyte function [5].

Adenosine deaminase (ADA) is a metalloenzyme that catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively and is implicated in purine metabolism [6,7]. So, it causes a reduction in the levels of adenosine. Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium, while it inhibits the effect of insulin on total hepatic glucose output. This suggests that adenosine causes local insulin resistance in the liver [8]. Adenosine is an agent that primarily decreases cyclic AMP (cAMP) accumulation, whereas insulin acts to inhibit lipolysis via a noncyclic AMP-dependent mechanism. Under appropriate conditions, one can see a marked synergism between the antilipolytic effects of insulin and adenosine. Fain JN et al. suggested that insulin cannot inhibit lipolysis due to high concentrations

of lipolytic agents unless cyclic AMP accumulation is maintained at low levels by adenosine [9]. ADA is widely distributed in human tissues with its highest activity in T-lymphocytes and is considered a good marker of cell-mediated immune response [10]. Adenosine on the other hand has been proven to modulate insulin action in various tissues. It reduces free fatty acid levels by its potent antilipolytic property and improves insulin sensitivity in adipose tissue [11]. As ADA is associated with T-lymphocyte activity, its altered blood levels may help in predicting immunological dysfunction associated with type 2 diabetes mellitus [12].

Some studies have explored the role of adenosine deaminase in insulin sensitivity and insulin resistance in T2DM among various populations. However, the association between serum insulin and ADA is far from clear in T2DM subjects. Thus in the current study, we aimed to estimate the altered levels of ADA and its correlation with insulin in patients with type 2 diabetes mellitus.

2. METHODOLOGY

A case-control study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur between October 2017 to September 2019. 40 diabetic patients were included as cases and 40 age, sex and, body mass index (BMI) matched healthy subjects as controls. The diabetic subjects were recruited from the outpatient clinic of Endocrinology, Department of Medicine, RIMS, while the control subjects were recruited from the subjects coming to the hospital for a routine health check-up and who were free from any systemic diseases. Both sexes were included.

2.1 Exclusion Criteria

1. Patients diagnosed with type 1 diabetes mellitus

2. Chronic kidney disease
3. Coronary artery disease and
4. COPD (chronic obstructive pulmonary disease) were excluded from this study.

P-value <0.05 was taken as statistically significant.

3. RESULTS

2.2 Sampling Technique

Overnight fasting venous blood of about 5 ml was collected from the anterior cubital vein of normal subjects and diabetic patients. About 2 ml of blood were collected in fluoride vial for blood glucose estimation and the remaining blood sample was collected in a sterile vial and allowed to centrifuge at 3000 rpm for 10 minutes to obtain serum and were used for the estimation of adenosine deaminase and insulin levels. Estimation of serum ADA has been carried out by the calorimetric method as described by Giusti G and Galanti B [13]. The estimation of fasting blood glucose was carried out by the Liquicolor Kit method based on the principles described by Brandam D and Trinder P [14]. Estimation of serum insulin was done by ELISA technique, using Calbiotech Human Insulin ELISA kit as explained by Tamas Csont [15].

2.3 Statistical Analysis

The collected data was analyzed using SPSS version 21 for windows. Descriptive statistics like mean, standard deviation, percentage and, proportion were used. T-test was used to test the differences between cases and controls on the demographic characteristics and biochemical profile of the subject. Pearson's correlation was applied to correlate serum ADA with serum insulin and fasting blood sugar.

The present study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal. The study included 40 cases of type 2 diabetes mellitus and 40 age and sex-matched healthy individuals. After thorough checking of the data, statistical analysis was done using SPSS version-21 software. The results and observations of the present study are being depicted as follows.

Table 1 shows no significant difference in age, sex distribution, systolic and, diastolic blood pressure while comparing T2DM and control subjects. BMI and fasting blood sugar levels were elevated in T2DM as compared to healthy controls and were statistically significant with * p-value < 0.05.

Table 2 shows that serum ADA levels (U/L) were significantly higher among cases with respect to controls (38.97±8.853 vs 20.05±5.309); p < 0.001). Serum insulin levels (µU/ml) were also increased in cases compared to controls and were statistically significant (18.08±5.554 vs 9.06±2.509; p <0.001).

Table 3 shows that there was a positive correlation (r = .014) between serum ADA level and serum fasting blood sugar among the cases but it was found that the relationship was statistically insignificant (p>0.05)

Table 1. Demographic and biochemical profile of the subjects

	Controls(n=40) (mean±SD)	Cases(n=40) (mean±SD)
Age (in years)	50.01±6.1	50.60±6.9
Sex (M/F)	21/19	22/18
BMI(kg/m ²)	21.62±2.106	24.84±3.004*
FBS(mg/dl)	88.22±7.731	183.22±55.719*
SBP(mm hg)	131.10±7.146	140.45±12.620
DBP(mm hg)	84.65±3.246	86.40±6.736

*p < 0.05 is considered to be statistically significant.

Table 2. Comparison of ADA and insulin in cases and control subjects

Parameter	Cases	Controls	P-value
Serum ADA(U/L)	38.97±8.853	20.05±5.309	<0.001
Insulin(µU/ml)	18.08±5.554	9.06±2.509	<0.001

Values are given in mean ± standard deviation and P < 0.001 is considered highly significant

Table 3. Correlation between serum ADA and serum fasting blood sugar among cases

Variable	Correlation coefficient (r)		p-value
	ADA	FBS	
ADA	1	0.014	0.931
FBS	0.014	1	0.931

P<0.001 is considered highly significant, r = Pearson's correlation coefficient

Table 4. Correlation between serum ADA level and serum Insulin level among the cases

Variable	Correlation coefficient (r)		p-value
	ADA	Insulin	
ADA	1	0.956	0.000
Insulin	0.956	1	0.000

P<0.001 is considered highly significant, r = Pearson's correlation coefficient

Table 4 shows that among the cases group, serum ADA were positively correlated with serum Insulin ($r= 0.956$, $p =0.000$) and since the p-value is < 0.001 , it is highly significant.

4. DISCUSSION

Diabetes is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [16]. T2DM is the predominant form of diabetes worldwide, accounting for 90% of cases globally [17]. Insulin deficiency leads to chronic hyperglycemia with disturbances of carbohydrates, fats and, protein metabolism [18]. Early identification of insulin resistance helps in minimizing the associated complications. Adenosine deaminase (ADA) is suggested to be an important enzyme for modulating the bioactivity of insulin [19]. ADA converts adenosine to inosine through an irreversible deaminase reaction. Adenosine is responsible for increasing glucose uptake into cells. Thus, higher ADA activity in insulin-sensitive tissue will decrease adenosine level which in turn decreases glucose uptake into cells [20].

In the present study, the mean serum adenosine deaminase levels among diabetic cases were higher than the controls. Based on Table 2 the mean value of ADA level in the study group was (38.97 ± 8.85) U/L. And the mean ADA level in controls was (20.05 ± 5.30) U/L. The value of mean ADA level in the present study and that of Khemka VK et al. are almost similar [12]. The mean ADA value in their study was found to be (38.77 ± 14.29) U/L in T2DM subjects versus (17.02 ± 5.74) U/L in controls. Also, this result is following the findings of Niraula A et al. [21]. In their study, the mean ADA level was found to be

(40.44 ± 17.97) U/L in T2DM cases and (10.55 ± 2.20) U/L in healthy controls. Both the study could show that the ADA level was found to be significantly higher in diabetes mellitus patients compared to healthy controls. Hoshino T et al. [22] suggested that high ADA levels in diabetes might be due to abnormal T-lymphocytes response or proliferation which in turn are due to defective secretion of insulin. This abnormal response or proliferation of T-lymphocytes' might be the mechanism that involves the release of ADA into the circulation of patients with diabetes mellitus.

In our study, the mean serum fasting blood sugar level was found to be significantly higher in diabetes cases compared to healthy controls $(183.22\pm 55.71$ vs $88.22\pm 7.73)$ mmol/L. In a study conducted by Ghazanfari Z et al. [23], it was found that fasting blood sugar was more reliable to separate diabetic from non-diabetic subjects compared to HbA1c (glycosylated hemoglobin). American Diabetes Association criteria for the diagnosis of diabetes 2019 includes FBS (fasting blood sugar) ≥ 126 mg/dL as one of the criteria [24]. On correlating fasting blood sugar with serum ADA in the study group, a positive correlation ($r=0.014$, $p=0.931$) was obtained (Table 3). This correlation was also observed by other researchers such as Sapkota LB et al. [25]. Hyperglycemia defines diabetes mellitus and is the cause of its most characteristic symptoms and long term complications. Hyperglycemia in T2DM occurs due to peripheral insulin resistance, declining β -cell function which eventually leads to β -cell failure. Obesity, diabetes and, physical inactivity are also major determinants of insulin resistance, hyperinsulinemia, hyperglycemia and, metabolic syndrome [26].

Also, coming to the findings of serum fasting insulin levels, the study could show that the insulin levels were significantly increased in diabetes mellitus compared to healthy controls (18.09 ± 5.55 vs 9.06 ± 2.50) $\mu\text{U/ml}$ respectively. Rao SN et al. [27] opined that fasting insulin levels determine the insulin resistance. Insulin resistance is a reduced physiological response of the peripheral tissues to the actions of insulin. Based on Table 4, a positive correlation was observed between serum ADA and serum insulin levels among the study group ($r=0.956$, $P=0.000$). The correlation is found to be statistically significant as the p-value is <0.001 . This finding is supported by the study of Shaikh SM et al. [28]. In their study, adenosine deaminase was positively correlated with serum insulin levels in diabetic cases ($r=0.302$, $P<0.001$). It was concluded from the study that serum ADA and serum insulin significantly raised in type 2 diabetes and correlated with each other and also with fasting blood glucose. ADA had a positive correlation with serum fasting blood sugar in the study on diabetes mellitus. ADA has been viewed as a parameter of interest in type 2 diabetes due to its role in oxidative stress, as a marker of cell-mediated immunity along with its effects on insulin by altering levels of adenosine. Therefore ADA can be used as an important parameter in the patients of type 2 diabetes mellitus.

5. CONCLUSION

It is evident from this study that serum ADA and serum insulin were significantly increased in type 2 diabetes mellitus and further, a strong positive correlation between ADA and insulin suggests an association between ADA and type 2 diabetes mellitus. Assessment of serum ADA level is cost-effective and the efficient use of this biomarker may help in establishing this enzyme as a good marker for assessing cell-mediated immunity in diabetic individuals. Thus, it was concluded that serum ADA may be used as a prognostic marker for the pathogenesis of T2DM and can also be implicated as a biomarker for predicting glycemic status in diabetic individuals. Larger prospective studies are required to fully assess the role of adenosine deaminase in the development and progression of type 2 diabetes mellitus.

CONSENT

A written informed consent from all the patient and control was obtained after a complete explanation of the study.

ETHICAL APPROVAL

The study was approved by the Research Ethics Board, Regional Institute of Medical Sciences, Imphal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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