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

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Abstract: ***Introduction: Diabetes mellitus is one of the most leading endocrinological disorder characterized by chronic hyperglycemia, metabolic abnormalities and long term complications. It is one of the most common non - communicable disease globally and fifth leading cause of death in most developed countries. Prevention of diabetes and its associated burden, primarily cardiovascular morbidity and mortality is a major health issue worldwide. Insulin is an important polypeptide hormone that regulate carbohydrate metabolism and with its receptor regulates blood glucose level. Adenosine deaminase (ADA) is a metalloenzyme that catalyses deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively. Serum ADA activity is seen to be increased with an increased in insulin resistance in diabetes mellitus patient. Also, the association between serum insulin and ADA in diabetes is not well understood. Objective: To estimate serum adenosine deaminase (ADA), fasting blood sugar level and serum insulin level among cases of type 2 diabetes mellitus and healthy controls and to correlate the levels of ADA and serum insulin levels among cases and controls. Methodology: A case control study, consisting of 80 subjects out of which 40 subjects diagnosed with T2DM and 40 healthy subjects, was carried out in the department of Biochemistry in collaboration with department of Medicine, RIMS, Imphal from October 2017 to January 2019. Serum ADA level, serum insulin and fasting blood glucose levels were measured. Result: The mean ± SD of serum insulin level in cases were significantly higher in case group (18.09±5.554 µIU/ml) when compared to control group (9.09 ±2.509µIU/ml). Also the mean serum ADA level in diabetic cases was higher in case group (38.97±8.853 U/L) and lower in the control group (20.05±5.309U/L). There was positive correlation between serum ADA and serum insulin level and was statistically significant as p < 0.05. Conclusion: Serum adenosine deaminase and serum insulin were significantly increased in type 2 diabetes mellitus than normal controls and correlated with each other.***

Keywords: Insulin, Adenosine deaminase, Diabetes mellitus

1. 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.1Diabetes is also said to be one of the most common non – communicable disease globally, and is the fifth leading cause of death in most developed countries.2 Currently, more than 400 million people suffer from diabetes worldwide of which type 2 diabetes mellitus makes up about 90%.3 Type 1 diabetes is predominantly due to auto - immune mediated destruction of pancreatic beta - cells and results in absolute insulin deficiency.4 Type 2 diabetes is a combination of resistance to insulin action and an inadequate insulin secretion.5 It is a leading cause of morbidity and mortality worldwide.

Adenosine deaminase (ADA) is a metalloenzyme that catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyadenosine to inosine and deoxyinosine respectively and is implicated in purine metabolism.6 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.7

Insulin is an important polypeptide hormone that regulates carbohydrate metabolism and coinjointly with its receptor regulates blood glucose level. Adenosine plays a crucial role in the bioactivity of insulin and regulates insulin activity in various tissues such as liver, myocardium, white adipose tissue and skeletal muscles.8 Adenosine increases glucose uptake inside the cells.9 Higher ADA activity will decrease adenosine levels and this in turn decreases glucose uptake into the cells. Serum ADA activity is seen to increase with an increased in insulin resistance in diabetes mellitus patient and may therefore be used as an effective tool in screening for insulin resistance and diabetes mellitus for which further studies is required. 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 and thus, the proposed study will focus on possible altered levels of ADA and its association with insulin in patients with T2DM.

2. Materials and Methods

This was a case control study carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal from October 2017 to September 2019. Forty diagnosed cases of type 2 diabetes mellitus attending medicine OPD and also admitted in the Medicine ward irrespective of sex, socio - economic status and ethnicity were enrolled as cases. Also, forty normal healthy individuals of comparable age and sex who were free from any systemic diseases were enrolled as control.

Exclusion criteria were as follows: Type 1 diabetes mellitus, Chronic kidney disease, Coronary artery diseases, COPD.

Written informed consent was obtained from all patients before sample collection. The study was approved by the Research Ethics Board, Institutional Ethics Committee (IEC), Regional Institute of Medical Sciences (RIMS), Imphal (Ref no. A/206/REB – Comm (SP) /RIMS/2015/270/13/2017

Laboratory measurements

Overnight fasting venous blood of about 5ml was collected from anterior cubital vein of normal subjects and diabetic patients. The blood samples were collected when the patients were presented for evaluation. About 2ml of blood were collected in fluoride vial for blood glucose estimation and the remaining blood sample was collected in sterile vial for estimation of adenosine deaminase as well as insulin. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Estimation of serum ADA was done by calorimetric method as described by Giusti G and Galanti B[10] Serum insulin was estimated by Calbiotech Human Insulin ELISA (Enzyme – Linked Immunosorbent Assay) kit.1[1] The estimation of fasting blood glucose was carried out by Liquicolor Kit method based on the principles as described by Brandam D and Trinder P.1[2]

Evaluation

Detail history regarding the duration of disease, age of onset of disease, associated symptoms of complications were recorded. Also, detailed socio – demographic and clinical characteristics were recorded for each patients including age, blood pressure, weight and height. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

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. Chi square test was used to test the association between proportions. P value < 0.05 was taken as statistically significant.

4. Results

Table 1: Distribution of the respondents by age among cases and controls

Age group in years	Cases n (%)	Controls n (%)	Total n (%)	Chi square test p value
>21 - 30	1 (2.5)	3 (7.5)	4 (5.0)	p = 0.050
>31 - 40	6 (15)	11 (27.5)	17 (21.3)	
>41 - 50	12 (30.0)	16 (40.0)	28 (35.0)	
>51 - 60	10 (25.0)	8 (20.0)	18 (22.5)	
61 and above	11 (27.5)	2 (5.0)	13 (16.2)	
TOTAL	40 (100)	40 (100)	80 (100)	

Table 1 shows age wise distribution of diabetic cases and controls. It is evident that the majority of type 2 diabetic cases (30%) occurred in the age group > 41 - 50 years, followed by 27.5% in the age group > 60 years, 25% in the age group of > 51 - 60 years, 15% in the age group of > 31 - 40 years and 2.5% in the age group of > 21 - 30 years. Among controls, majority of the respondents were in the age group of > 41 - 50 years (40%) followed by 27.5% in the age group of >31 - 40 years, 20% in the age group of > 51 - 60 years, 7.5% in the age group of > 21 - 30 years and 5% in the age group of > 60 years.

Table 2: Distribution of the respondents by gender among cases and controls

Gender	Cases n (%)	Controls n (%)	Total n (%)	Chi square test p value
Male	22 (55.0)	21 (52.5)	43 (53.8)	p = 0.823
Female	18 (45.0)	19 (47.5)	37 (46.2)	
Total	40 (100)	40 (100)	80 (100)	

Table 2 shows male predominance in both cases (55.0%) and in controls (52.5%) compared to 45.0% and 47.5% of female prevalence in cases and controls respectively.

Table 3: Distribution of the respondents by their site of dwelling

Site of dwelling	Cases (%)	Controls (%)	Chi square test p Value
Rural	13 (32.5)	17 (14.5)	p = 0.025
Urban	27 (67.5)	23 (57.5)	
Total	40 (100)	40 (100)	

Table 3 shows that in cases 13 (32.5%) belonged to rural areas and 27 (67.5%) belonged to urban areas. Among controls, 17 (14.5%) belonged to rural areas whereas 23 (57.5%) belonged to urban areas.

Table 4: Comparison of baseline, clinical and biochemical characteristics between cases and controls

Variables	Cases (N=40)	Controls (N=40)	p –value
BMI (kg/m^2)	24.84±3.004	21.62±2.106	< 0.001
SBP (mmHg)	140.45±12.620	131.10±7.146	< 0.001
DBP (mmHg)	86.40±6.736	84.65±3.246	0.142
FBS (mmol/L)	183.22±55.719	88.22±7.731	< 0.001
Insulin (µIU/ml)	18.09±5.554	9.06±2.509	< 0.001
ADA (U/L)	38.97±8.853	20.05±5.309	< 0.001

Table 4 shows that the mean BMI in cases was 24.84 kg/m^2 and standard deviation was 3.004. In controls, the mean BMI was 21.62 kg/m^2 with 2.106 as standard deviation. The BMI between the cases and controls were found to be statistically significant. ($p < 0.05$). The mean systolic and diastolic blood pressure in cases was 140.45 mmHg and 86.40 mmHg respectively. In controls, the mean SBP and DBP was

131.10 mmHg and 84.65 mmHg respectively. The systolic blood pressure between the two groups were found to be statistically significant (p< 0.05). However, there were no statistically significant difference between the two groups for diastolic blood pressure (p > 0.05). The mean fasting blood sugar in cases was 183.22 mmol/L with 55.719 as the standard deviation and for controls, the mean FBS was 88.22 mmol/L with a standard deviation of 7.731. They were found to be statistically significant. The mean serum insulin level in cases was 18.09 µIU/ml with a standard deviation of 5.554. Among the controls, the mean insulin level was 9.06 µIU/ml and 2.509 as standard deviation and they were statistically significant. The mean serum ADA level in cases and controls are 38.97U/L and 20.05 U/L with a standard deviation of 8.853 for cases and 5.309 for controls respectively and were statistically significant. It was found that the fasting blood sugar, the serum insulin level and serum ADA differed significantly among the two study groups. (p <0.05)

Table 5: Correlation between serum ADA level and serum insulin level among the cases

Parameter	Pearson Correlation	P – value
ADA	.956	.000

Table 5 shows that there was a positive correlation between serum ADA and serum insulin level and it was found to be statistically significant as p value < 0.05

5. Discussion

According to the American Diabetes Association, diabetes is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.1[3] Sinnott M et al [14] observed that type 2 diabetes has a long asymptomatic preclinical phase during which 20 - 30% of patients develop complications such as retinopathy, cardiovascular disease, neuropathy and nephropathy. Early detection followed by lifestyle modification and / or pharmacotherapy can delay or arrest disease progression.

Adenosine deaminase (ADA) is metalloenzyme that catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively and implicated in purine metabolism.6 It is an important enzyme for modulating the bioactivity of insulin.1[5] Adenosine is responsible for increasing glucose uptake into cells. Thus, higher ADA activity in insulin sensitive tissue will decrease adenosine level which in turn decrease glucose uptake into cells.1[6]

In the present study, 30% of T2DM cases were in the age group > 41 - 50 years, 27.5% in above 60 years, followed by 25 % in the age group > 51 - 60 years, 15 % in the age group of >31 - 40 years and 2.5% in the age group of > 21 - 30 years. Among the controls, 40% are in the age group of > 41 - 50%, 27.5% in the age group of >31 - 40 years, followed by 20% in > 51 - 60 years, 7.5% in > 21 - 30 years and 5% above 60 years.

In addition to obesity, age is one of the most important risk factors for type 2 diabetes mellitus. The prevalence of diabetes in our study is highest in the age group of > 41 - 50 years. This may be due to the fact that obesity is common among middle aged population. Sasai H et al [17] opined that the high prevalence of diabetes mellitus in middle aged population may be due to increased fat mass and reduced physical activity.

The present study shows male predominance in both cases and controls. Number of males is 22 (55%) and number of females is 18 (45%) in type 2 diabetes mellitus cases. Among the controls, 21 (52.5 %) were males and 19 (47.5%) were females. Among the controls, 21 (52.5%) were males and 19 (47.5%) were females. A study by Nordstrom Aetal [18] reported the occurrence of T2DM more in males (14.6%) than in females (9.1%).

Coming to the site of dwelling, the present study shows a higher occurrence of the disease in urban areas (67.5%) than the rural areas (32.5%). Zargar AH et al [19] also found a higher occurrence in urban areas (5.2%) compared to people from rural areas (4.0 %). The net effect of urbanisation is an epidemiological towards increasing rates of obesity and non – communicable diseases including diabetes mellitus.

The mean value of BMI in patients with T2DM was 24.84 kg/m^2 and the mean BMI in controls was 21.62 kg/m^2. In our study all the controls are in the category of healthy individuals. However, the BMI of the cases are beyond the healthy range. According to WHO recommendation, a BMI of 18.5 – 22 is considered healthy for the Asian population.2[0] A study by Natalia G et al [21] observed that higher than normal BMI were associated with an increased probability of being diagnosed with type 2 diabetes mellitus.

The present study shows a mean systolic and diastolic blood pressure of 140.45 mmHg and 86.40 mmHg respectively in diabetic cases. According to the study by Whelton PK et al [22] on guideline for high blood pressure in adults, it was observed that hypertension stage 1 is 130 - 139 mmHg SBP or 80 - 89 mm Hg DBP and hypertension stage 2 is ≥140 mmHg SBP or ≥ 90 mmHg DBP. Therefore, the present study could show that the diabetic cases under the study are hypertensive. Ferrannini E et al[23] in their study showed that elevated blood pressure values are a common finding in patients with T2DM and reflect the impact of the underlying insulin resistance on the vasculature and kidney.

In the present study, the mean serum adenosine deaminase levels among diabetic cases were higher than the controls. 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 [24] are almost similar. 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 in accordance with the findings of Niraula A et al.2[5] 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

In our study, the mean serum fasting blood sugar level was found to be significantly higher in diabetes cases compared to healthy. The observed mean value of FBS was (183.22±55.71) mmol/L in patients with T2DM and that of healthy controls was (88.22±7.73) mmol/L. In a study conducted by Ghazanfari Z et al [26], it was found that fasting blood sugar was more reliable to separate diabetic from non diabetic subjects compared to (HbA1c) glycosylated haemoglobin. American Diabetes Association criteria for diagnosis of diabetes 2019 includes FBS (fasting blood sugar) ≥ 126 mg/dL as one of the criteria.2[7]

Also, coming to the findings of serum fasting insulin levels, the present study could show that the insulin levels were significantly increased in diabetes mellitus compared to healthy controls. The mean serum insulin level was (18.09±5.55) µIU in study group v/s (9.06±2.50) µIU in controls. Rao SN et al[28] 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. It is important to identify the individuals who are at risk of insulin resistance for primary prevention. Features of metabolic syndrome are obesity, insulin resistance, dyslipidemia, impaired glucose tolerance and hypertension.

In the present study, a positive correlation was observed between serum ADA and serum insulin level among the study group (r_p = 0.956, p = 0.000). The correlation is found to be statistically significant as p value is < 0.001. This finding is supported by the study of Shaikh SM et al [29]. In their study, adenosine deaminase was positively correlated with serum insulin level in diabetic cases (r_p = 0.302, p< 0.005). 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 their 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.

6. Conclusion

It is evident from the present study that serum adenosine deaminase and serum insulin were significantly increased in type 2 diabetes mellitus and correlated with each other. Recently, the enzyme has been viewed as a parameter of interest in type 2 diabetes mellitus due to its role in oxidative stress, as a marker of cell mediated immunity along with its effects in insulin by altering levels of adenosine. Therefore, adenosine deaminase can be used as an important parameter in type 2 diabetes mellitus and also can be considered to reflect the glycemic status of the individual. Despite the relative small sample size, the present study provides evidence of the usefulness of the estimation of serum adenosine deaminase as a convenient biomarker for type 2 diabetes mellitus. However further study with large population may be required to fully understand the role of adenosine deaminase in the development and progression of type 2 diabetes mellitus.

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