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Original Article

Biochemistry Section

Altered Levels of Serum Adenosine Deaminase in Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is one of the leading not causes of increased morbidity and mortality in developing countries. Adenosine Deaminase (ADA) enzyme in purine metabolism catalyses the irreversible deamination of adenosine to inosine. Literature suggests that adenosine mimics the action of insulin and ADA reduces the adenosine levels, thus alters the intracellular glucose uptake. However, the role of ADA in Type 2 Diabetes Mellitus (T2DM) remains inconclusive.

Aim: To measure serum ADA levels in T2DM patients and analyse its correlation with the glycaemic status.

Materials and Methods: The case-control study included 54 clinically diagnosed T2DM subjects (aged 30-70 years) on oral hypoglycaemic treatment and 50 sex and age-matched apparently healthy individuals as controls. Serum Adenosine Deaminase (ADA), Fasting Blood Glucose (FBG), HbA1c, Lipid profile, {Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL)} along with anthropometric indices for BMI were measured. Descriptive statistical analysis was done using unpaired Students' t-test. Pearson correlation was used to analyse the correlation among various parameters. The p-value <0.05 was considered to be statistically significant.

Results: In our study, FBG, HbA1c, ADA, TC, TG were significantly increased in T2DM subjects as compared to control group (p-value <0.0001), Triglyceride (p-value <0.001). In contrast, HDL-C levels were significantly reduced (p-value <0.0001) in T2DM subjects compared to control group. BMI of the T2DM subjects was significantly higher (p-value <0.01) than control. A positive correlation between ADA and HbA1c (r -value 0.64: p-value<0.0001), and ADA and FBS (r value 0.48: p-value <0.001) was observed. However, no correlation was found between ADA and BMI (r value 0.01: p-value 0.09).

Conclusion: From the current study we propose that serum ADA can be used as a biomarker for predicting the glycaemic status of T2DM patient. It can also be used for assessment of dyslipidemia associated with diabetes.

Keywords: Dyslipidemia, Glycated haemoglobin, Hyperglycaemia, Insulin resistance

INTRODUCTION

Diabetes Mellitus (DM) is the most common heterogeneous disease prevailing world wide [1,2]. A chronic hyperglycaemia condition associated with disturbance in metabolism is characterised by decreased insulin secretion, insulin resistance or both [3]. In 2011, prevalence of diabetes was 366 million, which is predicted to increase to 552 million by 2030 [4]. Diabetes is emerging as a potential burden on developing country like India which needs to be tackled appropriately. One of the major risk factors for diabetes is obesity; however despite low number of overweight and obese individuals, India has an increased prevalence of diabetes indicating that diabetes may occur at a much lower BMI in Indians as compared to developed countries [5]. In all living systems, adenosine, an endogenous purine nucleoside act as a homeostatic regulator in skeletal muscle, pancreas and hepatic tissues via different pathways [6]. Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and skeletal muscle. It also acts as an anti-lipolytic agent and reduces free fatty acid levels, and thereby increases insulin sensitivity in target tissues [7]. The level of expression of adenosine nucleoside transporters and adenosine receptors has been shown to be affected in DM [8].

ADA is a metalloenzyme that catalyses the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively [1]. ADA is widely distributed in human tissues and highest activity is seen in T-lymphocytes. Functioning as both cytosolic enzyme and ectoenzyme, it is an essential protein in regulation of intracellular and extracellular adenosine levels in tissue [6]. Along with genetic and environmental factors, an interplay of immunological disturbances in diabetes with improper T-lymphocyte function and defect in insulin secretion or production contributes to pathophysiology of diabetes [9,10]. As ADA inhibits adenosine it is considered to be an important enzyme for modulating bioactivity of insulin and decreasing glucose uptake into cells [11-13]. ADA also leads to lipolysis that increases free fatty acid levels eventually leading to insulin resistance.

Poor glycaemic control in diabetes leads to long term glucotoxicity that results in micro and macro-vascular complications due to oxidative stress and further increases ADA levels [13].

In view of increasing burden of diabetes and association of adenosine with insulin this study was undertaken to evaluate the role of ADA in T2DM and to analyse its correlation with blood glucose level, glycaemic index and various biochemical parameters which may help in early detection of complication of diabetes.

MATERIALS AND METHODS

Study Population

This institution based case-control study was conducted over a period of three months (March 2018-May 2018) at The Oxford Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India. A total of 54 T2DM patients and 50 age-matched normal healthy subjects attending OPD for regular health check-up were selected as per convenient sampling with CI-95%.

Inclusion Criteria

A 54 clinically diagnosed type 2 diabetic cases (aged 30-70 years) (both newly diagnosed and known cases) on oral hypoglycaemic drugs were taken as cases. Age and sex-matched, 50 subjects who attended medicine OPD for regular health check-up were recruited as controls.

Pregnant women or individuals with acute/chronic liver or inflammatory disease, tuberculosis or renal disease, h/o hypertension were excluded from the study.

Ethical clearance was obtained from our Institutional Ethical Committee (Reg. No.: IEC/TOMCHRC/057/17-18) for the conduct of study. After obtaining an informed written consent from the participants, a detailed history and clinical examination was done. Anthropometric indices such as height, weight for BMI were measured.

Sample Collection

Overnight fasting blood samples were collected aseptically from the antecubital vein in a gel tube and allowed to clot. The samples were centrifuged at 2000 rpm for 10 minutes to obtain sera and samples were stored at -20ºC until further analysis. The biochemical parameters were analysed using fully automated analyser, ERBA 360.

Estimation of blood glucose was done by Glucose oxidase and peroxidase method [14]. HbA1c was measured by immunoturbidimetry method spectrophotometrically as per IFCC standardised reference method [15,16]. Total cholesterol was estimated by cholesterol esterase and peroxidase method, based on formulation by Allain CC et al., and modification of Roeschlau P et al., [17,18]. Triglyceride levels in the serum were estimated by Triglycerides-GPO Trinder method [19]. HDL was estimated by Trinder reaction [20].

ADA was assayed using ERBA 360 fully automated analyser and commercial kit (ERBA Manheim, code number 131933) as per manual's instructions.

STATISTICAL ANALYSIS

Descriptive statistical analysis of data was done. Results were expressed as mean±SD. Statistical analysis was done by unpaired student's t-test and correlation between parameters was assessed using Pearsons' correlation. The p-value<0.05 was considered statistically significant.

RESULTS

The biochemical parameters such as HbA1c and lipid profile (TC, TG, HDL) and serum ADA levels were estimated in both T2DM cases and control. The serum ADA, HbA1c, FBG, TC and TG levels were significantly higher in cases as compared to controls. HDL was found to be lower in cases as compared to controls (p-value <0.0001).

Obesity is considered an important risk factor in diabetes hence Body Mass Index (BMI) which is proposed as a useful marker for obesity was measured in both the groups. T2DM cases had significantly higher BMI as compared to control (p-value <0.01) [Table/Fig-1].

Variables	Cases (n=54)	Controls (n=50)	95% CI	p-value
Age (years)	54.2±11.3	51.5±10.4	-	0.2
FBG (mg/dL)	200.7±73.8	85±15.8	180.6-220.9	<0.0001*
HbA1c (%)	9.8±2.3	5.1±0.55	9.17-10.48	<0.0001*
ADA (U/L)	16±6.3	9.7±3.3	14.3-17.8	<0.0001*
TC (mg/dL)	190.5±16.8	174±15.9	185.9-195.1	<0.0001*
TG (mg/dL)	222.5±66.2	182.8±40.6	204.4-240.6	<0.001*
HDL (mg/dL)	40.9±8.6	49.9±6.7	38.53-43.27	<0.0001*
BMI (kg/m^2)	24±2.7	22.3±3.7	23.2-24.7	<0.01*

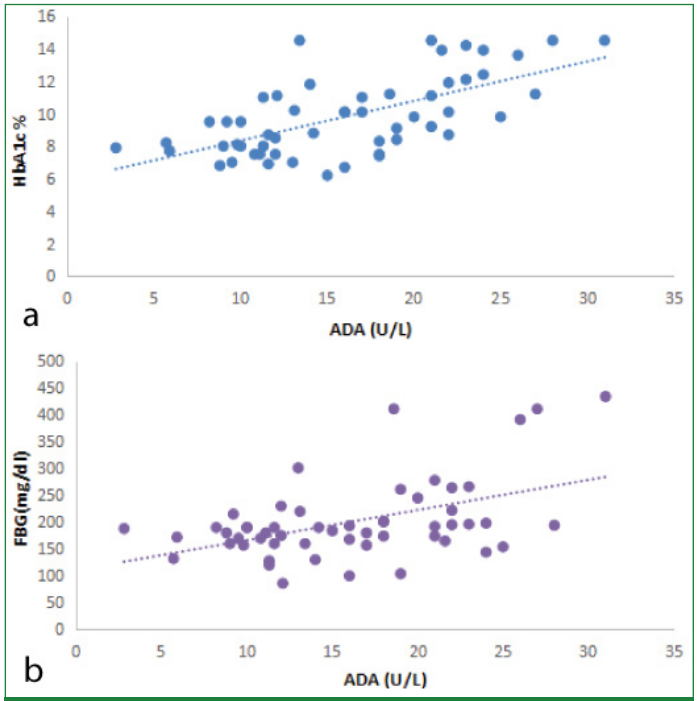
[Table/Fig-1]: Demographic and biochemical variables of the participants under study.
FBG: Fasting Blood Glucose; ADA-Adenosine Deaminase; TC-Total Cholesterol; TG-Triglyceride; HDL: High Density Lipoprotein; BMI-Body Mass Index. Results were expressed as Mean±SD; Unpaired students' t-test: *p-value<0.05 considered statistically significant

A positive correlation between ADA and TG (r-value 0.4; p-value <0.01) and negative correlation with HDL (r-value -0.3; p-value 0.02) was observed, however TC showed no correlation with ADA (r-value 0.2; p-value 0.1). Similarly, no correlation between BMI and ADA (r-value 0.01; p-value 0.9) was observed, since 63% of cases had normal BMI [Table/Fig-2].

A strong positive correlation was observed between ADA and HbA1c, (r-value 0.64; p-value <0.0001) [Table/Fig-3a] and ADA showed a positive correlation with FBG, (r-value 0.48; p-value <0.001) [Table/Fig-3b].

Variables	r-value	p-value
FBS Vs ADA	0.48	<0.001*
HbA1c Vs ADA	0.64	<0.0001*
TC Vs ADA	0.2	0.1
TG Vs ADA	0.4	<0.01*
HDL Vs ADA	-0.3	0.02*
BMI Vs ADA	0.01	0.9

[Table/Fig-2]: Correlation between serum ADA and various biochemical variables in T2DM cases.
Pearson correlation coefficient; *p-value<0.05 considered statistically significant



[Table/Fig-3]: Correlation between serum ADA and HbA1c (a) and FBG (b).

DISCUSSION

T2DM is characterised by chronic hyperglycaemia with altered metabolism and Insulin Resistance (IR). In order to maintain the normal glucose levels, the metabolic compensatory response to IR leads to hyperinsulinemia [21]. Identifying IR helps to cope with the disease and its complication at an early stage [7]. Due to IR, glucose and lipids are diverted to liver and adipose tissue, where they are utilized to form triacylglycerol that leads to hypertrophy of adipocytes which in turn causes cellular dysfunction, increased free fatty acids and proinflammatory state. Khemka VK et al., study have reported that metformin decreases IR and ADA levels thereby shows a positive correlation between ADA and IR [22]. Based on these findings, the present study was conducted to analyse the role of serum ADA in diabetics and its association with glycated haemoglobin.

In our study, ADA levels were observed to be significantly increased in diabetic cases. Our finding was in accordance with Kaur A et al., study that reported elevated ADA values in diabetic patients [2]. In contrary to our study, Shantaram M et al., showed a significantly decreased, ADA levels in diabetics who were undergoing diabetic treatment [23]. This possible decrease in ADA levels (ADA 2) can be due to the depressed cell mediated immunity in the patients undergoing treatment for diabetes.

In our study, we found a significant correlation between ADA and FBS which is similar to study by Almani SA et al., findings [8]. HbA1c also showed a strong correlation with ADA. Similar positive correlation was reported by Singh P et al., and Havilah P et al., [24,25]. The correlation between elevated ADA levels and HbA1c depicts the severity and chronicity of disease, since protein glycosylation is one

of the most common complications of T2DM. Overall, the increased serum ADA with HbA1c can be attributed to IR, and therefore can be a prognostic marker of disease [7].

Khemka VK et al., proposed that monitoring the ADA levels may give an account of insulin sensitivity and inflammation in non-obese cases [22]. In their study, they reported elevated ADA levels in non-obese T2DM cases, however no significant correlation was observed between ADA with BMI. Similarly, Nwankwo AA et al., proposed that ADA should be used in routine lab assessment of diabetes in both obese and non-obese individual [21]. The physiological activity of ADA is important in regulating the steady state concentration of adenosine which is an anti-inflammatory agent [26]. Chronic energy imbalance leads to production of large amount of inflammatory cytokines that interfers with insulin receptors' signalling cascade, the primary mechanism underlying the association between inflammation and glucose tolerance [27,28]. Adenosine increases the glucose uptake into cells by stimulating insulin activity via glucose transport, PDH activity and lipid synthesis. ADA impairs insulin sensitivity for glucose transport via downregulation of GLUT 4 receptors and results in anti-lipolysis by inactivating extracellular adenosine [7,13].

Adenosine is an inflammatory suppressant since it inhibits T-cell activation and proliferation. The progression of T2DM results in low grade inflammation that leads to pathogenesis of the disease [10]. Chronic hyperglycaemia leads to oxidative stress through enediol and superoxide ions formation and increases ADA levels [8]. Increased ADA in dyslipidemia has been reported by Nwankwo AA et al., [21]. In our study we found a positive correlation between TG and ADA and negative correlation between HDL and ADA suggesting the association of ADA with dyslipidemia. Havilah P et al., proposed that ADA can be considered an independent marker of glycaemic control [25]. The level of ADA increases with severity of the disease and with onset of associated complication. Therefore, it can be used as a simple and inexpensive biomarker to determine IR.

LIMITATION

Relatively small sample size. A larger prospective study will be required to establish the role of ADA in diabetics. In addition, serum insulin levels were not studied, and estimation of serum ADA in pre-diabetics and post glucose tolerance test could enhance the reliability of serum ADA in diabetics.

CONCLUSION

From the current study we conclude that serum ADA is positively correlated with HbA1c levels and therefore can be used as a biomarker for evaluation of glycaemic status of T2DM patients. It can be used for assessment of dyslipidemia associated with diabetics. Thus, ADA can be used in routine laboratory test for both obese and non-obese T2DM subjects.

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