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Elevated Adenosine Deaminase Activity to Glycated Hemoglobin and Lipid profile in Type 2 Diabetes Mellitus

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Abstract

Background: Adenosine has got insulin like activity on glucose and lipid metabolism particularly in skeletal muscles and adipose tissue. In view of increasing prevalence of Diabetes mellitus and mimicking action of adenosine with insulin we studied ADA as a new marker for detecting diabetes and its association with dyslipidemia and glycemic status (HbA1C) in Type -2 DM.

Materials and methods: A comparative cross sectional study was conducted in 40 type 2 diabetes mellitus patients attending diabetic clinic or admitted in the medicine ward for metabolic control of diabetes in Government medical college,Kozhikkode for a period of one year. ADA level in the serum is measured by endpoint method and glycated hemoglobin by ion exchange resin method. The correlation between ADA and HbA1c is calculated by Person correlation Coefficient with p- value <0.05,considered significant

Result: Among 40 diabetic patients mean ADA level in the serum is 38.56 ± 6.72 p<0.0001,and HbA1c is 9.28±1.5. Mean ADA level in the control group is 22.04 ± 4 and HbA1c is 4.95, ±0.56.

High ADA in cases show significant positive correlation (r =0.63, p<0.0001) with HbA1c.Positive correlation was also found between ADA with FBS, BMI, total cholesterol and LDL but it was not statistically significant.

Conclusion: Serum ADA could be used as biomarkers for assessing glycemic status in patients with type 2 DM. Also the diabetic patients had showed high serum total cholesterol, high triglyceride, and low HDL cholesterol than in controls, indicating that they are more prone for cardiovascular diseases.

Keywords: Adenosinedeaminase Glycated Hemoglobin Lipid profile Type2diabetes mellitus

Introduction

Type 2 diabetes mellitus is a chronic metabolic disorder, prevalence of which is increasing worldwide. The number of people affected expected to double in the next decade due to increase in ageing population, thereby adding to the already existing burden especially in poorly developed countries[1].The main pathogenic factor leading to hyperglycemia is the impaired regulation of hepatic glucose production, peripheral insulin resistance and declining β-cell function, eventually leading to β -cell failure. The primary events are supposed to be an initial deficit in insulin secretion and relative insulin deficiency in association with peripheral insulin resistance .As the disease progresses insulin secretion unable to maintain glucose homeostasis leading to hyperglycemia. In addition to hyperglycemia most of the Type 2 diabetic patients are characterized by obesity or predominant abdominal fat distribution. Adipose tissue promotes insulin resistance through various inflammatory mechanisms including release of free fatty acids and adipokine regulation[2].The pro-

inflammatory cytokines like Tumor Necrosis Factors-alpha ,Interleukin-1- beta,Interleukin-6,numerous chemokines, epigenetic factors, other transcriptional and metabolic pathways are also induce low-grade tissue specific inflammatory responses leading insulin resistance. Moreover, chronic exposure of pro-inflammatory mediators stimulates the activation of cytokine signaling proteins which ultimately block the activation of insulin signaling receptors in β-cells of pancreatic islets [3]

The action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium is mimicked by the purine nucleoside adenosine. In adipose tissue adenosine signaling results in enhanced insulin sensitivity measured by the ability of insulin to inhibit lipolysis. The mechanism by which adenosine enhances insulin sensitivity and inhibits lipolysis is mediated by the cAMP- dependent protein kinase activation cascade. Insulin inhibits activation of hormone sensitive lipase by norepinephrine or glucagon. In human adipocytes, insulin inhibits the lipolysis by nor epinephrine more efficiently when adenosine is present. Similar to adenosine, activation of rat adipocyte-A_1adenosine receptor by pharmacological agents showed the same effect on improving insulin sensitivity and consequent inhibition of lipolysis. Green et al, reported a 30% reduction in the responsiveness to insulin in adipocytes using pharmacological reagents selective for A_1- adenosine receptor, as prolonged treatment impaired insulin dependent lipolysis.[4]

To identify the average plasma glucose concentration over a longer period of time a form of hemoglobin chemically linked to the sugar named glycated hemoglobin is used. In diabetes mellitus increased levels of glycated hemoglobin have been associated with nephropathy, retinopathy and cardiovascular disease [5]. During the normal 120-day life span of the red blood cell, glucose molecules react with hemoglobin forming glycated hemoglobin. Once the hemoglobin molecule is glycated, it remains in this form. Red blood cells (RBCs) that contain the hemoglobin circulate iin the bloodstream for three to four months before being broken down and replaced. During that time, the RBC can bond irreversibly to glucose in the bloodstream. A buildup of glycated hemoglobin within the red blood cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle. Thus, A1C readings higher than about 6% indicate higher than normal amounts of glucose roaming the blood stream in the past 120 days. Other studies state that the major proportion of its value is related to a rather shorter period of two to four weeks. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long term serum glucose regulation. In individuals with poorly controlled diabetes, the quantities of this glycated hemoglobin are much higher than in healthy people[6]

In mammalian tissues adenosine level is regulated by two utilizing enzymes adenosine kinase and adenosine deaminase and producing enzyme 5'nucleotidase. ADA catalyses the irreversible hydrolytic deamination of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine respectively[7].An increase in ADA activity in T2DM patients has been reported in several researches. Elevated ADA levels are also found in obesity, metabolic syndrome, liver cirrhosis and hepatoma, TB, brucellosis, typhoid fever, hypoxic states and cell mediated immune responses. However, it is difficult to conclude whether changes in ADA activity are the cause or result of actual insulin resistance[8]. In the present study, we measured serum ADA activity inT2DM patients to evaluate the relationship between serum ADA activity with glycemic status and lipid profile in T2DM patients

Materials And Methods

A Comparative cross sectional study was conducted

Inclusion criteria : Study was conducted in patients attending diabetic clinic or admitted in the medicine ward for metabolic. control of diabetes in medical college, Calicut from January 2011 to January 2012. Two study groups are selected. Ethical approval of study was obtained

Group 1

40 consecutive type 2 diabetic patients including both male and female, of age group 30-60 years

Group 11

30 non-diabetic subjects in the age group 30-60 years from bystanders of other patients or from medical or paramedical staff. Diabetes mellitus was diagnosed on the basis of FBS≥126mg/dl or a patient already on anti diabetic medication.

Exclusion Criteria

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Patients not giving written consent, individuals with diabetic complications, those with hypertension, those who are pregnant, subjects with history of infectious or alcoholic hepatitis, chronic renal disease, coronary artery disease , disease affecting immune system like rheumatoid arthritis, cancers like leukemia, chronic infections like tuberculosis, nephrotic syndrome are excludedfromthestudy

Estimation Of Serum ADA

Serum adenosine deaminase was estimated by colorimetric method of giusti & galanti[9]

Principle: Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue colored indophenol complex formed is directly proportional to the amount of ADA present in the sample.

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$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \xrightarrow{\text{Alkaline Medium}} \text{Blue IndophenolComplex}$$

Glycated hemoglobin was estimated by ion exchange resin method[10]

Serum cholesterol was determined by end point estimation using cholesterol esterase and peroxidase[11]

HDL is solubilised by a special detergent; other lipoproteins such as LDL, VLDL and chylomicrons are not disrupted. After HDL is selectively disrupted, HDL cholesterol is measured enzymatically.

From the values of total cholesterol, HDL and triglyceride, the value of LDL was determined by using the Friedewald's formulae[12].

LDL Cholesterol=Total cholesterol- (HDL-C+TG/5)

VLDL cholesterol is estimated from the total amount of Triacyl glycerol. VLDL = [Triacylglycerol] /5

Results

The results were expressed as mean and standard deviation .The statistical significance of the differences between the values was assessed by ANOVA. A significantly higher ($p<0.001$) mean values of body mass index (BMI), fasting blood sugar (FBS), glycated hemoglobin (HbA1c), and lipid profiles except high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein (LDL) were found in type 2 diabetic cases compared with controls. Serum ADA activities were significantly higher in cases compared with controls ($p<0.0001$) showing significant positive correlation ($p<0.0001$) with HbA1c.Positive correlation was also found between ADA with FBS, BMI, total cholesterol and LDL but it was not statistically significant.

Discussion

Serum ADA activities were significantly higher in type 2 diabetic patients compared with controls showing significant positive correlation with HbA1C.ADA modulates concentration of adenosine which has got insulin like activity on glucose &lipid metabolism in skeletal muscle and adipose tissue. Thus Serum ADA can be used as a biomarker for assessing glycemic status in patients with type 2 DM. Similar findings were supported by many other studies performed before. Bhavita Patel *et al*[13] showed that with an increase in blood glucose & HbA1c levels serum ADA levels also increases and this may play an important role in determining the glycemic status in patients with Type-2 diabetes Mellitus. Debes Ray *et al*[14] also supported these

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findings.Vineet Kumar *et al*[15] observed increased level of serum ADA in type 2 diabetic patients and suggested the role of ADA in modulating the bioactivity of insulin. Supriya *et al*[16] states that increased ADA level can be used to determine the glycemic status in the patients of type 2 DM and serve as a marker for insulin resistance. Raised ADA levels can be an early indicator of progressive diabetic change and help to take preventive measures for the development of diabetic complication and thereby improving the outcome of the disease.

In the present study dyslipidemia was an obvious feature among the study group.TG and VLDL were significantly elevated in the study group when compared to controls. HDL was reduced among diabetics when compared to nondiabetics. Similar findings could be seen in *J. Prathyusha Rao et al* [17]where he documented increase in lipoprotein(a) also . Apart from an increase in triglycerides and VLDL and decrease in HDL, it was observed that total cholesterol was also found to be raised . Similar findings were supported by V Siva Prabodh *et al* [18]. Shanmuga Priya, *et al*[19] and G. Bhambhani, G.D *et al* [20]. The combination of high TG, low HDL, and central obesity are the hall marks of the metabolic syndrome, which occurs in most of the people with diabetes mellitus. This group of patients is more prone for premature death from heart disease. It is supported by Venkateswarlu *et al* [21] where statistically significant association can be seen between the elevated total cholesterol and insulin resistance. Also an attempt has been made by Obaidullah *et al* [22] to evaluate the risk factors for coronary heart disease in DM patients. Early detection of all these risk factors can prevent or delay coronary artery disease in diabetic patients where ADA can be used as a biomarker for glycemic and lipemic index

Conclusion

Type-2 DM is a major public health problem worldwide. The incidence and prevalence of diabetes is increasing at an alarming rate. We found that the highest ADA levels were present in poorly controlled type-2 DM patients who had the highest HbA1c.The diabetic patients had a higher prevalence of high serum total cholesterol, high triglyceride, high VLDL-C, and low HDL-C than in controls, indicating that diabetic patients were more prone for cardiovascular diseases. These findings clearly show that serum Adenosine deaminase reflects the glycemic status in the diabetic individual, indicating a possible involvement of adenosine in the diabetic processes.

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Table 1 Correlation of serum ADA activity with various biochemical parameters in 40 type 2 diabetes patients//control

Biochemical parameters		ADA activity Control	Cases
HbA1c	r value	0.362	0.63
	P value	0.05	0.0001
FBS	r value	0.191	0.219
	P value	0.311	0.174

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BMI	r value	-0.089	0.072
	P value	0.641	0.657
TCL	r value	0.096	0.003
	P value	0.616	0.987
HDL-C	r value	0.173	-0.17
	P value	0.359	0.916
LDL-C	r value	0.056	0.009
	P value	0.770	0.956
VLDL-C	r value	-0.105	-0.38
P value		0.580	0.814
TG	r value	-0.123	-0.095
	P value	0.516	0.056

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