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SERUM ADENOSINE DEAMINASE ACTIVITY: A POSSIBLE EXPLANATION FOR HYPERACTIVE IMMUNE STATUS AND ONSET OF NEPHROPATHY IN DIABETIC SUBJECTS

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Abstract: Diabetes Mellitus is a disorder which although is classified as a disorder of the endocrine system ,but the ground reality is that it is a complex syndrome which involves multiple organ systems of the body including the nervous system, the kidneys & the eye to name a few. The incidence of this disorder has been progressively increasing in the past few decades. It is estimated that by the year 2030, 438 million of the adult population will be having diabetes. Adenosine Deaminase (ADA) is an enzyme of purine metabolism. Our study has revealed that serum ADA levels are substantially raised in diabetic patients with no complications whereas patients who have diabetic nephropathy seem to exhibit normal serum ADA levels. This finding is indicative of the fact that the increased susceptibility of diabetics to bacterial & fungal infections might not be due to a deficient immune status. Our study suggests that the hyperglycemia in type 2 diabetes has a strong correlation with ADA activity, which in turn is suggestive of the fact that hyperglycemia plays a role in activation of T lymphocytes in diabetics & hence the high ADA activity. The nephropathy probably results due to an autoimmune reaction to abnormal modified glycated proteins which result from the persistent hyperglycemia.

Keywords: Diabetes Mellitus, Adenosine Deaminase, Immune Status, Diabetic Nephropathy

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INTRODUCTION

WITH HUMILITY AND REVERENCE WE THE AUTHORS HEREBY DEDICATE THIS TO THE LOTUS FEET OF "Late. Prof. Dr. Seema Das."

Diabetes is often found to be coexisting with obesity.Obesity as a cause for diabetes has been proved beyond doubt. Obesity is associated with a greater risk of cardiovascular diseases, kidney diseases, liver & gall bladder diseases.[1] The spectra of pathogenesis in diabetes mellitus is characterised predominantly by two hallmark features:-

a) Insulin resistance, defined herein as inability of insulin to suppress hepatic glucose output and to promote peripheral glucose disposal.[3]

b) Compromised ß cell secretory activity resulting in a failure to secrete adequate amount of insulin to overcome the insulin resistance.[2,3]

A patient suffering from type 2 diabetes almost invariably presents with a breakdown in lipid dynamics which results in an elevation in levels of free fatty acids & triglycerides in blood. This ofcourse is in addition to the classical feature of hyperglycemia experienced by all patients of type 2 diabetes.[2]

Adenosine deaminase (ADA) is an enzyme involved in the metabolism of purine nucleosides, catalyses the irreversible hydrolytic deamination of adenosine (Ado) and 2´-deoxyadenosine (2´-dAdo) to inosine and 2´-deoxyinosine, respectively.[3]Studies have shown that ADA which reduces adenosine levels, increases basal and noradrenaline stimulated lipolysis in adipocytes.[4,5]

Adenosine's role as an anti lipolytic agent & its role in lowering levels of free fatty acids has been identified.[1]

Several studies have demonstrated elevated levels of adenosine deaminase in individuals with type 2 diabetes mellitus, but the exact pathogenic role of elevated ADA activity in type 2 DM remains to be elucidated.[6,7,8,9]

Insulin administration has been shown to reduce the elevated ADA levels in type 2 diabetics.[6]

Adenosine Deaminase exerts its effects predominantly by regulating the concentration of intracellular and extracellular adenosine. Conditions which lead to elevated adenosine formation and release (e.g. hypoxia) have been shown to increase the expression of ADA.[10]

Adenosine actions are multiple and it acts through its receptors following release from the cell. The A1 receptor is the only adenosine receptor expressed in the adipose tissue, and acting

through this receptor adenosine exerts potent anti-lipolytic effects. In fact, A1 receptor agonists have been shown to decrease free fatty acid levels and increase insulin sensitivity.[1]

Adenosine, acting through its receptors also affects multiple tissue and organ functions including pancreas, liver, kidneys, skeletal muscle, heart, vascular tissue etc.The expression level of adenosine nucleoside transporters and adenosine receptors has been shown to be different in diabetes.[11,12,13,14]

A gross imbalance in energy dynamics of the body occurs in diabetes mellitus. Chronicity of this energy imbalance results in mitochondrial dysfunction, endoplasmic reticulum stress & hypertrophy of adipocytes.[15] These hypertrophied adipocytes release a large amount of inflammatory cytokines. Immune cells in close proximity of the adipocytes infiltrate the adipose tissue easily & the consequence is the insulin resistance associated with this inflammation resulting finally into type 2 diabetes.

Adenosine has been shown to be a non-redundant endogenous regulator of many different functions in the immune system. Hence, the adenosine receptors can also be of importance as drug targets in the adipose tissue to suppress the underlying inflammation in obesity and thereby increase insulin sensitivity. In addition, the A2B receptor has been reported to mediate effects in the immune system of rodents that can protect against the development of type 1diabetes, which is an autoimmune disease. A2A receptor agonists have been reported to elicit wound healing and anti-inflammatory effects that can be useful for treating diabetic neuropathic foot ulcers.[1]

Highest ADA activity has been reported in lymphoid tissues,skeletal muscle & heart.[16]

Adenosine has been proved to be responsible for glucose uptake in the cells.[17] Therefore in an insulin sensitive tissue,if ADA activity is high it will lead to depletion of adenosine & consequently the glucose uptake in the cells will be compromised.ADA is a key player involved in lymphocytic proliferation & differentiation.T-lymphocytes have been found to posses high ADA activity.[18] Thus an inference may be drawn that if ADA activity in insulin sensitive tissues is suppressed,it may facilitate glucose uptake in the cells resulting in a better utilisation of glucose at the cellular level.

MATERIALS & METHODS

STUDY DESIGN:-

This case control study was carried out over a period of 6 months in Dept of Biochemistry at IMS & SUM Hospital.The subjects for the study were catagorised into three groups.

Group A:- This group comprised of 20 age and sex matched healthy individuals who were not having NIDDM or any of its associated complications.They did not have any history of major illness at the time of examination.

Group B:-This group included 20 patients who were suffering from NIDDM since last 5-7 years and were not having any diabetic nephropathy as was revealed by serum creatinine ,blood urea & urine examination.

Group C:- This group included 20 patients who were suffering from NIDDM since 10 years or more and were known cases of diabetic nephropathy.

Subjects in group B & C were selected from the patients who presented to the outpatient department of endocrinology,IMS & SUM Hospital,Bhubaneswar.Subjects in group A or the so called controlled group were recruited from amongst the people who presented to the department for routine health checkup.

A written informed consent from the patients and controls 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, obesity, 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 ethical committee at IMS & SUM Hospital which follows the Helsinki guidelines. The blood samples of the subjects for analysis was sent to a private lab in kolkata namely CHIKITSA MEDICARE Pvt Ltd.

METHOD:-

7 ml of venous blood was collected with full aseptic precautions after 12 hours of fasting.2ml of this blood sample was collected in a fluorinated vacutainer for estimation of fasting blood sugar. Rest 5 ml was collected in a plain vacutainer which was processed ultimately to obtain serum. This was used for analysing serum ADA & creatinine.

GLUCOSE ESTIMATION

Fasting blood glucose estimation was done by GODPOD {Glucose oxidase peroxidise method}.This is an enzymatic method employed in the clinical laboratory for the estimation of glucose.Glucose is oxidized by glucose oxidase to gluconic acid and H_2O_2 is liberated. The colorimetric indicator, quinonemine is generated from 4 – amino antipyrene and phenol by H_2O_2 under the catalytic action of peroxidise. Intensity of colour generated is directly proportional to glucose concentration. Normal range in serum or plasma is 70-100 mg/dl.

ADA ESTIMATION

ADA estimation was done by a commercially available kit by TULIP DIAGNOSTICS PVT LTD, Goa, which is based on the method described by Giusti & Galanti.[19] Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form blue indophenols complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indo phenol complex formed is directly proportional to the amount of ADA present in the sample.

REFERENCE RANGE :-

BODY FLUIDS	NORMAL	STRONG SUSPECT	SUSPECT POSITIVE	POSITIVE
SERUM/PLASMA	<30 units/litre	30-40 units/litre	>40-60 units/litre	>60 units/litre
PLEURAL FLUID	<30 units/litre	30-40 units/litre	>40-60 units/litre	>60 units/litre
PERICARDIAL FLUID	<30 units/litre	30-40 units/litre	>40-60 units/litre	>60 units/litre
ASCITIC FLUID	<30 units/litre	30-40 units/litre	>40-60 units/litre	>60 units/litre
CSF	≤ 10 units/litre			>10 units/liter

SERUM CREATININE ESTIMATION

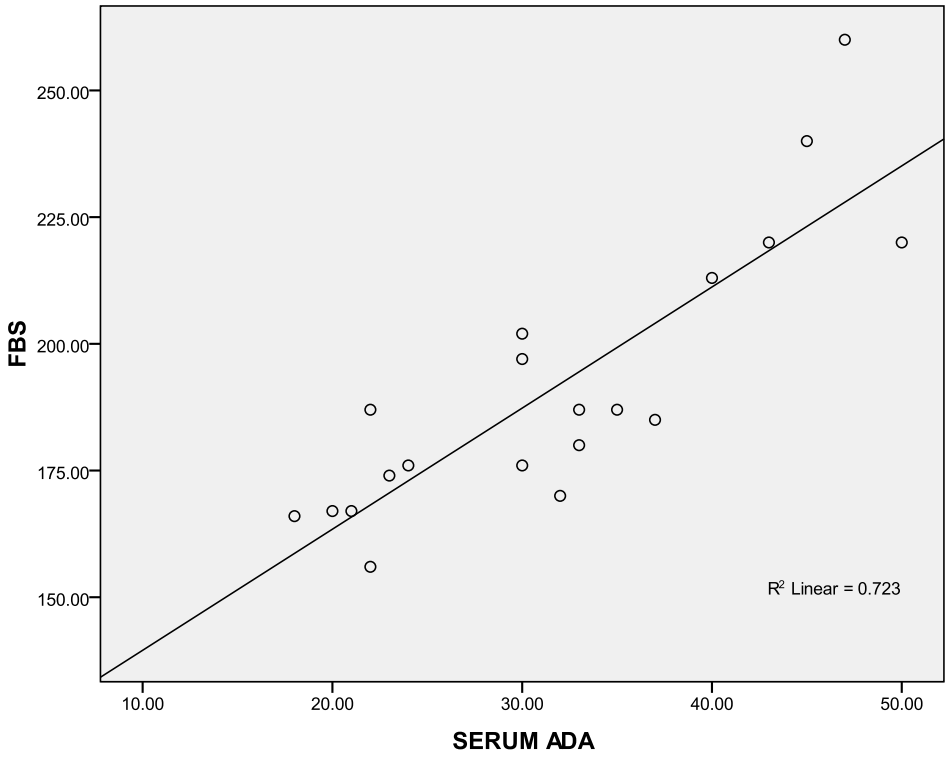
Serum creatinine estimation was done by Jaffe's kinetic method without deproteinization. Creatinine forms a yellow orange compound in alkaline solution with picric acid. At a low concentration of picric acid as used in this method, precipitation of protein does not take place. As a result of rapid reaction between creatinine and picric acid, the secondary reactions do not cause interference. Normal range of serum creatinine is 0.7-1.0 mg/dl.

RESULTS & DISCUSSION

Our study revealed that probably the predisposition of diabetics to suffer from infections & complications such as nephropathy has a multifactorial etiology.

Table 1:-Table showing data (expressed as MEAN±SD) of the three groups of subjects involved in the study.

	GROUP A(Healthy controls)	GROUP B(Diabetics without nephropathy)	GROUP C(Diabetics with nephropathy)
Fasting blood sugar(FBS)	83.1500±7.54129	191.5000±27.01559	262.00±72.72804
Serum creatinine	0.7400±0.12312	0.7800±0.14364	3.9950±1.43508
Serum ADA	13.4500±2.99956	31.7500±9.61290	16.7000±3.84023



'r' VALUE: -0.85

'p' VALUE: <0.01(SIGNIFICANT)

Fig:-Scatter diagram showing correlation between FBS & SERUM ADA in diabetics without complication(GROUP B).

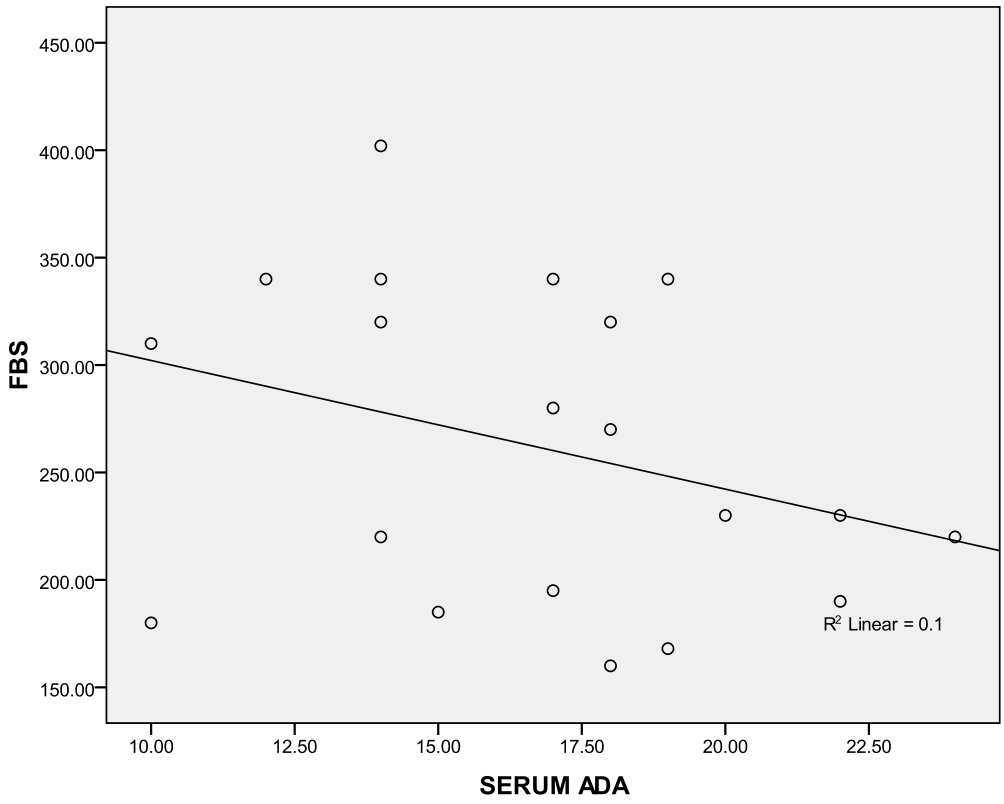


Fig:-Scatter diagram showing no correlation between FBS & SERUM ADA in diabetics with nephropathy (GROUP C).

From the above tables it is quite evident that chronic diabetic patients who had developed nephropathy exhibited raised levels of both serum creatinine as well as fasting blood glucose. It has been proved beyond doubt by other different biochemical & clinical studies that poor glycemic control in diabetics was responsible for aggravation of the disease process & hence more complications. Serum ADA activity was found to be normal in diabetics with nephropathy just like the control group. However in diabetics without nephropathy serum ADA level was found to be higher than normal. This finding corroborate with findings reported by Jose D Mendez etal in 2010 & Raisa Faheem etal in 2013 respectively[20,21] . Thus an inference can be drawn that persistent hyperglycemia in diabetes causes increased T-lymphocyte activity and hence the higher ADA levels. Thus it may be concluded that susceptibility of diabetics to suffer from infections is probably not due to a deficient immune status. The nephropathy and other microvascular complications might be the result of an autoimmune response to the abnormally modified glycoproteins that are formed due to the persisitent hyperglycemia[20,21].

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