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SERUM ADENOSINE DEAMINASE ACTIVITY- A PROMISING GLYCEMIC MARKER IN UNCOMPLICATED TYPE 2 DIABETES MELLITUS

DR. A. PODDAR[1], PROF. DR. S. DAS[2], DR.B. SINGH[3], DR. A. K. SAHOO[4], DR. A. K. RANA[5], DR. D. D. BEHERA[6]

1. PGT Biochemistry, IMS & SUM Hospital, Bhubaneswar, Orissa.
2. Professor, Dept of Biochemistry, IMS & SUM Hospital, Bhubaneswar, Orissa.
3. Associate Professor, Dept of Biochemistry, IMS & SUM Hospital, Bhubaneswar, Orissa.
4. Associate Professor, Dept of Endocrinology, IMS & SUM Hospital, Bhubaneswar, Orissa.
5. PGT Biochemistry, IMS & SUM Hospital, Bhubaneswar, Orissa.
6. Assistant Professor, Hitech Medical College & Hospital, Bhubaneswar, Orissa.

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Abstract: Diabetes is a disease characterized primarily by hyperglycemia. The hyperglycemia associated with diabetes is just one of the symptoms among the vast constellation of symptoms it exhibits. The major challenge faced by physicians & endocrinologists while treating diabetic patients is to achieve a control over the exorbitant blood glucose levels which eventually leads to the various micro vascular & macro vascular complications associated with diabetes. To achieve the same, the patients are treated with oral ant diabetic drugs & insulin. The blood glucose levels & glycosylated haemoglobin (glycemic marker) are monitored at regular intervals to follow up the glycemic status of the patient. Adenosine Deaminase is an enzyme of purine metabolism. Adenosine is known to exert potent metabolic effects by its receptors. Association of adenosine deaminase activity with activated T lymphocyte population is proved beyond doubt by various biochemical studies. It is presently a biochemical marker for chronic inflammatory states such as tuberculosis. Due to close association of adenosine deaminase in glucose metabolism, a study of its enzymatic activity in diabetes would further help in understanding the pathogenesis of the complex spectra of symptoms associated with diabetes. The purpose of our study was to monitor adenosine deaminase activity in patients with type 2 diabetes mellitus who do not have any symptoms of any micro vascular or macro vascular complication of diabetes & to observe whether any correlation exists between serum fasting blood glucose levels & ADA activity as well as between serum HbA1c levels & ADA activity.

Keywords: Adenosine deaminase, Diabetes mellitus, type 2 diabetes, Glycemic marker, Adenosine.

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Corresponding Author: DR. A. PODDAR

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INTRODUCTION

"IN LOVING MEMORY OF DR. SEEMA DAS WHO WILL ALWAYS REMAIN IN OUR HEARTS FOR HER OVERWHELMING AFFECTION"

Diabetes is often found associated with obesity which in turn has been implicated with increased risk of cardiovascular diseases,kidney diseases & gall bladder diseases[1] .A jeopardy in lipid metabolism is very often found to be associated with diabetes which eventually results in increased levels of free fatty acids & triglycerides in blood[2] .Adenosine's role as an antilipolytic agent has been well established & its role in decreasing free fatty acid levels has been identified[1] .Multiple metabolic effects of adenosine have been identified which it exerts through its receptors[1] .The A_1 receptor of adenosine is exclusively expressed in the adipose tissue & through this receptor adenosine exerts its antilipolytic effects[1] .A_1 receptor agonists have been demonstrated to lower free fatty acid levels in blood & thus increase insulin sensitivity[1] .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]

Various biochemical studies in the past have reported elevation of adenosine deaminase levels in serum of patients suffering from type 2 diabetes mellitus,but the exact reason for the elevation of the same needs to be elucidated[6,7,8,9] .Insulin administration has been shown to reduce ADA levels in diabetics[6] .

Conditions such as hypoxia which lead to overproduction of adenosine act as an inducer for over expression of ADA & this eventually explains the mechanism of action of ADA,which is by regulating the levels of intracellular & extracellular adenosine[10] .

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 is a case control study carried out at IMS & SUM Hospital ,Bhubaneswar over a period of 6 months.The subjects for the study were catagorised into 2 groups :-

Group A:--This group comprised of 20 patients with history of type 2 diabetes mellitus since last 2-3 years who presented to the endocrinology opd at IMS & SUM Hospital.These patients were being treated with oral antidiabetic drugs & did not have any symptoms of microvascular or macrovascular complications of diabetes.

Group B:-- This group comprised of 20 age and sex matched healthy subjects,who presented to the institution for regular health checkup.This group served as the control group.

A written consent was obtained from all the cases & controls after proper explanation of the procedure.Approval was taken from the institutional ethical committee at IMS & SUM HOSPITAL which follows the Helsinki guidelines.The blood samples drawn from the patients were sent to a private lab in kolkata namely CHIKITSA MEDICARE PVT Ltd.The statistical data was analysed with SPSS v17.0 .

INCLUSION CRITERIA :-

i)Patients who are known cases of type 2 diabetes mellitus with no obvious symptoms of complications.

ii)Age>35 years & < 65 years

iii)Patient being treated with oral antidiabetic drugs.

iv)No H/O tuberculosis,psoriasis,viral heptitis,rheumatoid arthritis.

EXCLUSION CRITERIA :-

i)Diabetic patients who have symptoms of obvious complications of diabetes.

ii)H/o rheumatoid arthritis,viral hepatitis,psoriasis,tuberculosis.

iii)Diabetics on insulin therapy.

iv)Pregnant women.

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 .

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.

Normal reference range:

Serum :-	Normal	<30 u/l
	Strong suspect	30 u – 40 u/l.
	Suspect positive	>40 u/l – 60 ul.
	Positive	>60 u/l.

RESULTS & DISCUSSION

Adenosine deaminase activity was found to be elevated in the diabetic patients as compared to the healthy controls in our study.Increased adenosine deaminase activity would thus lead to depletion of adenosine.Adenosine through its receptors exerts potent metabolic effects.[1] A_1 receptor agonists have been found to be associated with increased insulin sensitivity.[1] Thus,depletion of adenosine due to increased adenosine deaminase activity would mean increase in insulin resistance in the body & subsequent hyperglycemia,which is a hallmark feature of diabetes mellitus.This explains the hyperglycemia encountered in diabetic patients.

Our study also reveals a very strong correlation between fasting blood glucose levels & serum ADA activity in patients with uncomplicated type 2 diabetes.A similar correlation also exists between serum ADA levels & HbA1c.HbA1c is considered to be an established glycemic marker used to asses the glycemic status of a diabetic patient & also forms the diagnostic criteria for diabetes mellitus(HbA1c>6.5 gm%).Since a strong positive correlation exists between fasting blood glucose levels ,HbA1c & serum ADA,therefore estimation of serum ADA might serve as an inexpensive glycemic marker for assesing the glycemic status of a diabetic patient on oral antidiabetic agents who is free from symptoms of microvascular & macrovascular complications of diabetes & also chronic inflammatory conditions in which ADA activity tends to flare up.The statistical data and other pictorial representations of the same are hereby depicted below:-

Data for Group A

FBS	SERUM ADA	Hba1C
167	15	7.0
185	18	7.4
165	17	6.8
220	25	8.4
200	20	7.0
178	19	6.8
189	20	7.0

168	17	7.2
148	15	6.8
210	21	7.9
235	21	8.2
189	19	7.3
176	18	6.5
192	19	7.0
190	17	7.1
165	15	6.8
193	19	7.3
182	17	6.9
176	17	6.7
180	18	7.0

MEAN:-

1.FBS- 185.4000±20.22089

2.SERUM ADA-18.3500±2.39022

3.HbA1C-7.1550±0.49362

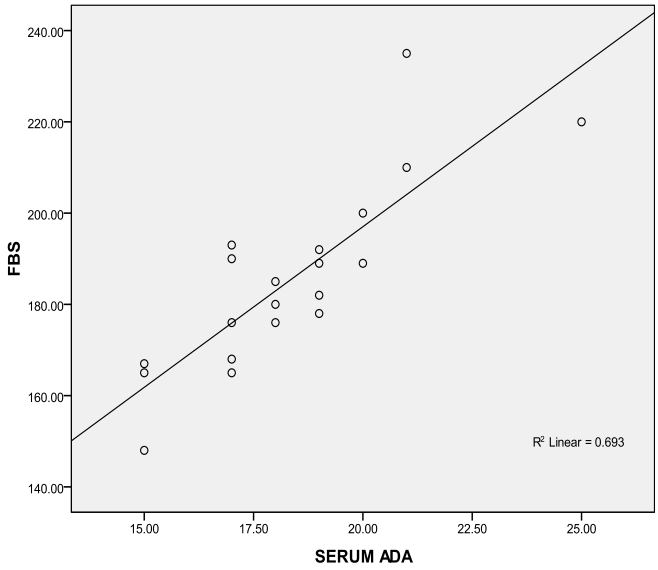

Fig 1:-Scatter plot showing correlation between fasting blood sugar levels & serum ADA levels in patients with uncomplicated type 2 diabetes mellitus.(Group A).

'r' VALUE: -0.832

'p' VALUE: <0.01(SIGNIFICANT)

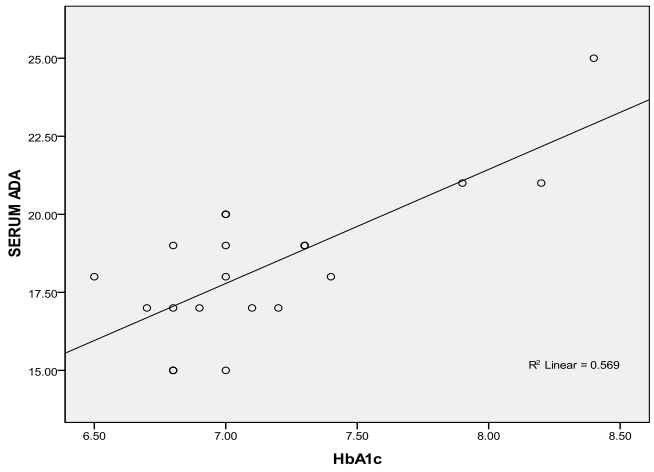

Fig 2:-Scatter plot showing correlation between serum ADA levels & HbA1c in patients with uncomplicated type 2 diabetes mellitus.(Group A).

'r' VALUE: -0.755

'p' VALUE: <0.01(SIGNIFICANT)

Data for Group B (Controls)

FBS	SERUM ADA	Hba1C
80	5	5.0
76	8	6.1
74	9	5.3
70	7	5.0
69	6	5.6
79	9	5.3
96	9	4.7
90	8	6.0
84	8	5.2
90	9	5.3
69	8	5.0
88	10	6.0
84	9	5.8
90	9	6.1
75	6	4.6
77	8	6.1
72	7	5.8
71	7	5.7
70	9	5.2
73	8	5.1

MEAN:-

1.FBS- 78.8500±8.42474

2.SERUM ADA-7.9500±1.27630

3.HbA1C-5.4450±0.48175

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