Correlation of Adenosine Deaminase Activity with Glycated Hemoglobin Levels in Type II Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by hyperglycemia which results from defects in insulin secretion, insulin action or most commonly both. The chronic hyperglycemia and attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves and blood vessels.

Materials and methods: The present study includes 90 subjects, of which 60 were cases of type 2 DM from Department of Endocrinology, Osmania General Hospital. Investigations were performed at the Department of Biochemistry, Osmania Medical College/ Osmania General Hospital; Hyderabad and results were analyzed.

Results: A total of 90 patients were recruited for the study which included 30 Type II Diabetes mellitus patients with glycated hemoglobin ≥ 7 %, 30 Type II Diabetes mellitus with glycated hemoglobin < 7% and 30 healthy individuals as controls. The following parameters were analyzed.1. Fasting plasma glucose, 2.Glycated hemoglobin (HbA1C), 3.Serum Adenosine deaminase, 4. Serum uric acid

The data was analyzed using Graph Pad Prism software version 7.0. Descriptive results are expressed as mean and SD of various parameters in different groups.

Conclusions: There is clear cut elevation of serum ADA in Type 2 Diabetes mellitus. But due to short half life and diurnal variations of ADA, to establish ADA as a routine diagnostic and prognostic marker in the laboratory, substantial number of samples have to be analyzed in larger and more elaborate studies.

Keywords: Fasting blood glucose, Hyperglycemia, Insulin, Metabolic dysregulation, Uric acid.

INTRODUCTION

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by hyperglycemia which results from defects in insulin secretion, insulin action or most commonly both. The chronic hyperglycemia and attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves and blood vessels.[1]

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people.[2]

According to Diabetes Atlas 2015, published by the International Diabetes Federation, the number of people with diabetes in India is around 69.2 million and is expected to rise to 123.5 million by 2040.[3]

Diabetes mellitus is characterized by chronic hyperglycemia resulting from diverse etiologies, environmental and genetic factors acting together. The long term control of type 2 diabetes mellitus is judged by glycosylated hemoglobin which was first isolated by Allen et al.[4] The levels of HbA1c are increased in diabetic patients and reflects their metabolic control over the past 8-10 weeks.[5]

Adenosine deaminase, an enzyme, which is present in red cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2‘- deoxyadenosine to 2‘-deoxyinosine. Inosine and 2‘-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid.[6]

ADA is considered as a good marker of cell mediated immunity.[7] High lymphocyte ADA activities were found to be elevated in diseases in which there is cell mediated immune response.[8]

A significant correlation between the ADA levels and uric acid levels in diabetes was analyzed by various researchers. They concluded that high uric acid levels in DM patients were due to the increased ADA activity.[9]

While some investigators[10] reported the association of high uric acid levels with Type 2 DM, others[11] demonstrated low uric acid levels in diabetic patients.

ADA is reported to be an important enzyme for modulating the bioactivity of insulin, but its clinical significance in Type 2 DM is yet to be characterized.

Even though there are reports available on serum Adenosine deaminase levels and serum Uric acid levels in patients of Type 2 diabetes mellitus but these are still not very clear and conclusive. Moreover, study showing correlation of serum

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ADA activity and serum uric acid levels with the glycemic control in Type 2 DM patients have not been conducted in this part of the state yet.

Hence, in the light of the above mentioned facts, the present study was designed to evaluate the serum ADA activity and serum uric acid levels in patients of Type 2 diabetes mellitus and its comparison with the controls and further to find any correlation of serum ADA activity and serum uric acid levels with the glycemic control in patients of Type 2 diabetes mellitus.

MATERIALS AND METHODS

Setting: A case control study was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad.

Source of samples and data:

The present study includes 90 subjects, of which 60 were cases of type 2 DM from Department of Endocrinology, Osmania General Hospital. Investigations were performed at the Department of Biochemistry, Osmania Medical College/ OsmaniaGeneral Hospital; Hyderabad.

In the present study the individuals included in the study were divided into 3 groups**.** (Table 1.)

All the subjects were in the 40-65 years of age group and of either sex. Informed oral consent was taken from all individuals who took part in the study.

Inclusion criteria:

Group 1 includes age and sex matched healthy subjects.

Group 2 includes patients with type 2 DM with HbA1c level <7%

Group 3 includes patients with type 2 DM with HbA1c level ≥7%

Exclusion criteria:

Individuals with

- Type 1 diabetes mellitus
- High (>30 g/d) alcohol consumption
- Known liver diseases
- Known gastrointestinal diseases
- On corticosteroids, methotrexate, amiodarone, tamoxifen or other hepatotoxic drugs
- Chronic infection like tuberculosis, sarcoidosis
- Gout, Rheumatoid arthritis
- Hemolytic anemia

Specimen collection:

Fasting venous blood samples were collected from all groups. 3ml of blood was collected into serum vacutainer (red cap), 2ml into sodium fluoride vacutainer (grey cap)and 2ml into EDTA tubes (purple cap).

Parameters Estimated:

Serum: 1) Serum ADA 2) Serum Uric acid

Plasma: 1) Fasting Plasma Glucose

EDTA blood: 1) Hb% 2) HbA1c%

The ethical issues involved in this study were reviewed and approved by the ethics scientific committee of Osmania Medical College.

The data was analyzed using Graph Pad prism software version 7.0. Descriptive results are expressed as mean and SD of various parameters in different groups.

PLASMA GLUCOSE

Method: Trinder's Method.(GOD-POD)[12]

Principle: Glucose Oxidase (GOD) oxidizes Glucose to Gluconic Acid and Hydrogen Peroxide. In presence of enzyme Peroxidase, released Hydrogen Peroxide is coupled with 4-Hydroxy benzoic acid (HBA) and 4-Amino antipyrine(4-AAP) to form colored Quinoneimine dye.

Glucose oxidase

β-D glucose + H_2O + O_2 → Gluconic acid + H_2O_2

Peroxidase

H_2O_2 + 4HBA + 4AAP → Quinoneimine dye + 2 H_2O

The intensity of pink color formed is proportional to glucose concentration and can be measured photometrically between 490-550 nm.

GLUCOSE STANDARD 100 mg/dL (5.55 mmol/L)

GLUCOSE STANDARD 400 mg/dL (22.2 mmol/L)

Reference Ranges:

Normal Values: Fasting : 70-100 mg/dL

Postprandial: <140 mg/dL

Serum adenosine deaminase

Method:

PNP-XOD/ Kinetic method or enzymatic Non-Giusti method[13,14]

Assay Principle ADA Assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD).

H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

Adenosine +H_2O → Inosine + NH_3

Inosine +Pi → Hypoxanthine + Ribose1 phosphate

Hypoxanthine + 2H_2O +$2O_2$→ Uric acid + 2H_2O_2

H_2O_2 + 4AAP + EHSPT → 2H_2O + Quinone dye (λ max 546nm)

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per minute at 37ºC.

Serum uric acid

Method: Uricase –Trinder, End point method [15]

Principle:

This reagent is based on Trinder reaction. The series of reactions involved in the assay system is as follows:

Uric Acid + O_2 + H_2O Uricase Allantoin + CO_2 + H_2O_2

TOOS + 4AAP + 2H_2O Peroxidase Quinoneimine dye + 4H_2O

1. Uric acid is oxidised to allantoin by uricase with the

production of H2O2

2. The peroxide reacts with 4-aminoantipyrine (4-AAP) and TOOS in the presence of peroxidase to yield a quinoneimine dye. The absorbance of this dye at 546 nm is proportional to uric acid concentration in the sample.

Reference Values in Serum
Adults Male: 3.5 – 7.2 mg/dl
Adults Female: 2.6 – 6.0 mg/dl

Linearity limit:
Upto 20 mg/dL. For higher values the samples should be diluted with Normal saline and the results multiplied with dilution factor.

Interferences: Following substances do not interfere: hemoglobin up to 500 mg/dL, bilirubin up to 16 mg/dL, triglyceride up to 2000 mg/dL.

Quality Control:
For Quality Control Erba Norm and Erba Path were used. HbA1c %

Method: Ion Exchange Resin Method [16]

Principle: Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation exchange resin; the non-glycosylated hemoglobin binds to the resin leaving GHb free in the supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

Reference values: [17]
Nondiabetes: 4.0-5.6%
Prediabetes: 5.7-6.4%
Diabetes: >6.4%

RESULTS

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad.

A total of 90 patients were recruited for the study which included 30 T2DM patients with HbA1c ≥ 7 %, 30 T2DM with HbA1c < 7% and 30 healthy individuals as controls.

The following parameters were analyzed.

1. Fasting plasma glucose
2. HbA1c
3. Serum Adenosine deaminase
4. Serum uric acid

The data was analyzed using Graph Pad Prism software version 7.0. Descriptive results are expressed as mean and SD of various parameters in different groups.

The results were expressed in milligrams /deciliter for Fasting plasma glucose and serum uric acid, U/L for ADA. Hb and HbA1c are expressed as percentages (%).

The Mean ± SD of all the parameters studied in the total cases were significantly different from those of controls.

Mean value of FPG (Graph 1) was highest in Group 3 followed by Group 2 and Group 1. Mean value of HbA1c (Graph 2) was highest in Group 3 followed by Group 2 and Group 1.

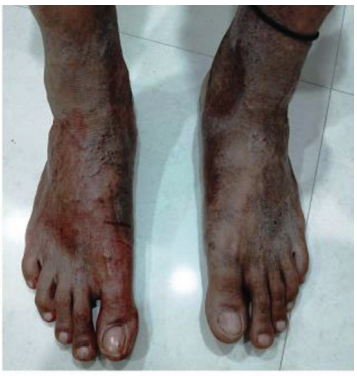
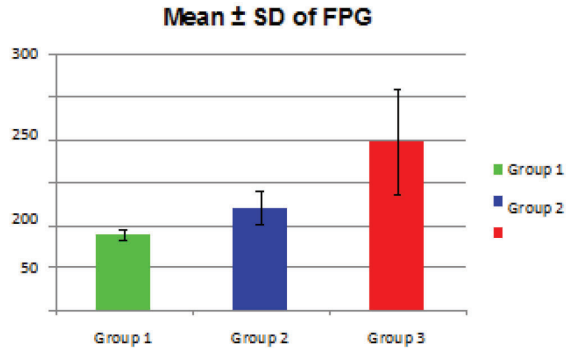
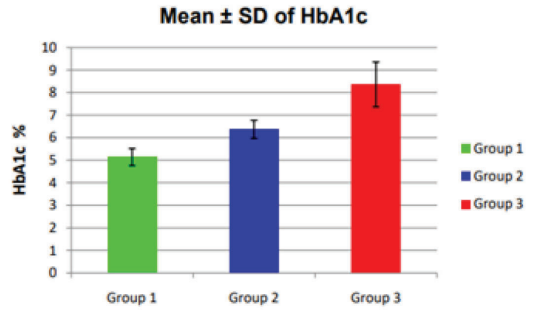


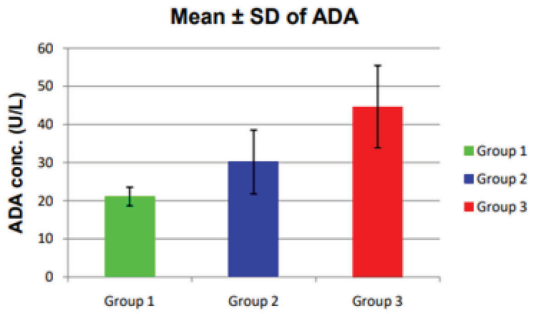
Figure 1: External appearance of feet in Diabetes mellitus type II



Graph 1: Graphical Representation of Mean ± SD of FPG in the 3 groups



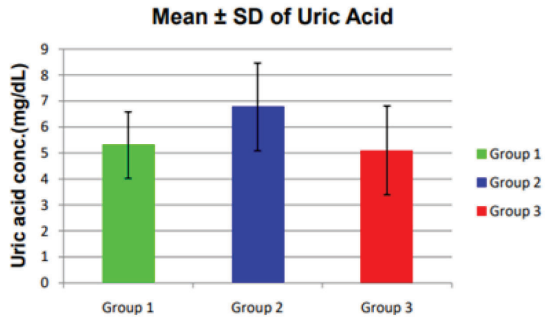
Graph 2: Graphical Representation of Mean ± SD of HbA1c in the 3 groups



Graph 3: Graphical Representation of Mean ± SD of serum ADA in the 3 groups

Mean value of serum ADA (Graph 3) was highest in Group 3 followed by Group 2 and Group 1

Mean value of serum uric acid was highest in Group 2 showing a bell shaped correlation with HbA1C(Graph 4)



Graph 4: Graphical Representation of Mean ± SD of Serum Uric acid in the 3 groups

DISCUSSION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[18] (Figure 1) The incidence and the prevalence of Type 2 DM is globally increasing and becoming a major public health problem for health care providers.[19]

The importance of early diagnosis is to reduce diabetes complications.[20]

The major clinical complications of T2DM are the result of persistent hyperglycemia which leads to numerous pathophysiological consequences such as diabetic retinopathy, peripheral neuropathy, poor wound healing, renal failure, and erectile dysfunction.. In addition Type-2 diabetes mellitus has been shown to be a state of increased free radical activity.[21] Chronic hyperglycemic status favors auto-oxidation and the formation of advanced glycation end products. The generation of free radicals in the diabetic patients can be due to the following mechanisms.[22]

Hyperglycemia leads to activation of NADPH oxidase, which is a multi-subunit enzyme, that catalyses O^- formation by one electron reduction of O2 using NADPH or NADH as electron donor.[22]

$2O_2$ +NADPH (or NADH) → $2O^-_2$ + NADP (or NAD) + H

Another source of superoxide anion formation could be auto-oxidation of glucose which is subjected to enediol rearrangements that result in the formation of an enediol radical ion. This species is capable of reducing molecular oxygen to form superoxide anion.[23]

Hyperglycaemia causes formation of Advanced Glycation End Products (AGEs) as a result of non-enzymatic reactions between intra-cellular glucose-derived dicarbonyl precursors with the amino group of both intracellular and extracellular proteins.[24]The AGEs stimulate receptors for advanced glycation end products (RAGE). Their interaction is believed to initiate and aggravate the diabetic complications. In addition they increase the generation of reactive oxygen species in macrophages thereby causing heightened oxidative stress[25] AGEs bind to AGE receptors on several celltypes (endothelial cells, mesangial cells and macrophages) lead to release of cytokines;

TNF-α, IL-1, IL-6 and growth factor from macrophages and mesangial cells resulting in activation of T lymphocytes.[26]

The limitations of the study were**:** Serum transaminase and serum insulin levels which are known to be related to ADA were not included in the study.

Studies have found a better sensitivity, specificity, and positive predictive value for postprandial glucose than fasting plasma glucose. Postprandial glucose, which was a better correlate and accurately predicts HbA1c value, could have been included in the study

CONCLUSIONS

The present study was carried out in the Department of Biochemistry, Osmania Medical College/Osmania General Hospital.

90 individuals were included in the study, who were divided into 3 groups eachgroup consisting of thirty individuals.

1. Group 1 consisted of healthy individuals.
2. Group 2 consisted of patients with type 2 DM with HbA1c level < 7%
3. Group 3 consisted of patients with type 2 DM with HbA1c level ≥ 7%

The following parameters were analyzed in all groups: FPG, HbA1c, Serum ADAactivity, and Serum Uric acid levels.

All the three parameters, FBS, HbA1c and ADA levels were found to be increased in the patients of Type 2 DM as compared to controls.

The mean FBS levels of Group B and Group C were found to be significantly higherthan Group A ($p < 0.001$).

The levels of ADA were significantly higher in both Group B and Group C as comparedto Group A ($p<0.001$)

The mean serum uric acid levels of Group B were significantly higher than Group C andGroup A ($p<0.001$)

In this study there is significant hyperglycemia in the cases when compared to thecontrols. Significantly higher values of ADA in cases compared to controls suggest that ADAplays a role in the pathophysiology of type 2 DM and its complications.

A positive correlation of ADA level with good and poor glycemic control suggests its important role in modulating the bioactivity of insulin. Thus, increased ADA activity mightbe a marker for insulin resistance.

Serum ADA level increased with increase in HbA1c. Therefore estimation of serum ADA might serve as a glycemic marker for assessing the glycemic status of type 2 diabetes mellitus patients.

The serum UA levels were found increased with moderately increasing levels of HbA1c (<7%) and then decreased with further increasing levels of HbA1c (>7%). Serum Uric acid showed a bell shaped relation with HbA1c.

Serum ADA and serum uric acid levels reflect closely related components of the same disease, Type 2 Diabetes Mellitus.

There is clear cut elevation of serum ADA in Type 2 Diabetes mellitus. But due to short half life and diurnal variations of ADA, to establish ADA as a routine diagnostic and prognostic marker in the laboratory, substantial number of samples has

to be analyzed in larger and more elaborate studies.

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