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Correlation of Glycated Albumin Levels with Serum Adenosine Deaminase in type 2 Diabetes Patients

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Abstract
Background: *In recent times Glycated Albumin routinely using as glycemic parameter to asses the status of diabetes complications. Type 2 diabetes mainly due to shortage or scarcity of insulin secretion. This insulin secretion again influenced by many factors and enzymes in our body, one such enzyme is Adenosine Deaminase (ADA). So far there are very limited studies available to explore the clinical correlation between glycated products such as Glycated Albumin with ADA in type 2 diabetes mellitus (Type 2 DM).*

Material and Methods: *In this case control study, all the subjects included were divided into 3 groups. Group A consisted of 80 normal healthy individuals who served as controls with no history of DM. Group B consisted of 80 patients of Type 2 DM both males & females in the age group of 45-70 years on oral hypoglycaemic drugs with GA<18%. Group C consisted of 60 patients of Type 2 diabetes mellitus both males & females in the age group of 45-70 years on oral hypoglycaemic drugs with GA>18%. serum levels of fasting blood sugar, glycated albumin, HbA1$_c$, ADA and uric acid were estimated in all the subjects.*

Results: *All the three parameters, FBS, GA, HbA1cand ADA levels were found to be increased in the patients of Type 2 DM as compared to controls.*

Conclusion: *From the Present Study, It Is Concluded That There Is an Increase In Serum ADA Levels With Increase In GA levels.*

Keywords: *Glycated Albumin (GA), HbA1$_c$, adenosine Deaminase, (ADA), Fasting Blood Glucose (FBG), Type 2 Diabetes Mellitus (T2DM).*

Introduction

Worldwide around 170 million people are affected by Diabetes mellitus (DM) especially in developing countries[1]. DM is characterized by low levels of insulin either in its release or action and causes hyperglycemia in turn disturbs the carbohydrate, lipid, and protein metabolisms[2].To the oxidative stress, hyperglycemia is one of the reason which forms free radicals and superoxide ions further they elevates adenosine deaminase (ADA) activity [3]. The deamination of adenosine to inosine catalyzed by ADA, which is a purine metabolizing enzyme and controll's intracellular and extracellular adenosine concentration[4].

Several studies showed that ADA has a key role in insulin secretion as well as in glycemic control as adenosine influnces insulin activity via several processes such as transport of glucose into the cells, activity of pyruvate dehydrogenase, synthesis of lipids, oxidation of leucine and cyclic nucleotide phosphodiesterase activity[5].

Therefore, in Type 2 DM, ADA role is very importantas a marker for prognosis of its complications. Previous literatures showed thatthe high levels of ADA in Type 2 DM cases and also the administration of insulin has been shown that it decreased the elevated ADA activity in type 2 diabetics[6,7]. Insulin sensitivity for glucose transport and antilipolysis by inactivating extracellular adenosine, influenced by ADA as adipocytes release it spontaneously. Lipolysis activated by ADA concentration via high cAMP accumulation due to noradrenaline[8]. The enzyme Adenosine deaminase, present in RBC 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 [9].The short term control of diabaetes mellitus can be monitored by measuring Glycated albumin (GA) is on monthly basis. Albumin is the most common protein found in serum, forming approximately an 80% concentration of the circulating blood plasma protein. It is replaced in the body approximately every 20-25 days. As with other proteins in the body, it is subject to glycation by excess sugar. A comparison with total albumin provides a simple, stable index of glycationover a three week to one month [10-12]. The long-term control of diabetes mellitus is judged by glycosylated haemoglobin which was first isolated by Allen et al [13].

Materials and Methods

A total of 240 people included in this study (160type 2 diabetic patients and 80 healthy controls) attending the Diabetic O.P.D., NIMS Medical College & Hospital, Shobha Nagar, Jaipur Rajasthan, India. Ethical clearance was obtained from the institutional ethical committee (Regd. No. NIMS/IEC/64/2017) as well as oral informed consents were obtained from the subjects. The present study was conducted from July 2017 to December 20117. These 240 subjects were divided into 3 groups:

- GROUP A comprised of 80 normal healthy individuals both males and females in the age group of 45-70 years from the general population who volunteered for getting included in the present study.
- GROUP B comprised of 00 patients of Type 2 Diabetes Mellitus both males & females in the age group of 45-70 years on oral hypoglycaemic drugs with GA <18 %.
- GROUP C comprised of 60 patients of Type 2 Diabetes Mellitus age and sex matched on oral hypoglycaemic drugs with GA >18 %.

Blood samples were collected from all the subjects after at least 8 hours fasting. Blood specimens were collected into EDTA tubes for GA, HbA1c and in Serum Separator Tube for fasting glucose (FPG) and uric acid. All the biochemical analysis was performed using an Hitachi 902. The plasma glucose was estimated by GOD-POD method using a Olympus autoanalyzer In the fasting sample. In addition to plasma glucose, Glycated albumin and HbA1c were measured. Plasma GA levels were measured by an enzymatic method by using albumin specific protease, ketoamine oxidase and an albumin assay reagent on a Hitachi Auto analyser (Lucica GA-L, Asahi Kasei Pharma Corp, Tokyo, Japan). HbA1C was estimated by high-performance liquid chromatography (Bio-Rad) according to International Federation of Clinical Chemistry (IFCC) and transferable to Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP). ADA estimation in serum by using ADA MTB diagnostic lit from Microxpress (Tulip diagnostic ltd).

Statistical Analysis

The data were statistically evaluated by SPSS

statistical package version 16.0. Independent sample t-test (2-tailed) was used to compare means of different parameters. Pearson's correlation test was performed to examine various correlations. The value of GA and HbA1c was given as percentage of total albumin and total hemoglobin (%) and values of all other parameters were given in mg/dl. All values are expressed as mean ± standard error of mean. The results were considered significant when $P < 0.05$.

Results

After careful clinical examination and confirmed diagnosis by diabetologist, 160 patients presenting type 2 diabetes mellitus were included in the study. The biochemical investigations were carried out in the blood samples of patients and age and sex matched healthy controls. The Demographical data of enrolled cases are given in Table 1.

Table 1: Characteristics of patients and control groups

| Demographical parameters		Control(80 n)(Mean ± SD)	Type 2 Diabetes cases(180 n) (Mean ± SD)
Sex(Male/Female)		55/25	135/45
Age in Years	Male	45±6.42	56±7.34
	Female	48±3.25	58±4.62
BMI		24±1.8	25±2.14
Duration of Diabetes in years		-	7.25±3.75
Smoking in number		-	35
Alchoholics in number		-	25
Hypertension in number		-	42

The mean FBS levels of Group A were 5 ± 0.50 mmol/l, Group B were 7.2 ± 0.80 mmol/l and the corresponding values among Group C subjects were 8.3 ± 2.1 mmol/l. In the present study, the mean FBS levels of Group B and Group C were found to be highly significant than Group A (p <0.001). Although the mean FBS levels of Group C were higher than Group B but the difference was statistically not significant (p=0.80). It was further observed that the mean GA and HbA1$_c$ levels in Group A were 14 ± 2 and 5 ± 1 %. In

Group B were 16 ± 4 and 6 ± 1.56% and the corresponding values among Group C were 20 ± 4 and 8 ± 0.5%. From this study it was observed that the difference in the levels of GA and HbA1$_c$ was found to be insignificant between Group B and Group A (p=325 and p= 0.310) Table-2


Figure-1 Correlation of FBS,HbA1c,GA in all the three groups
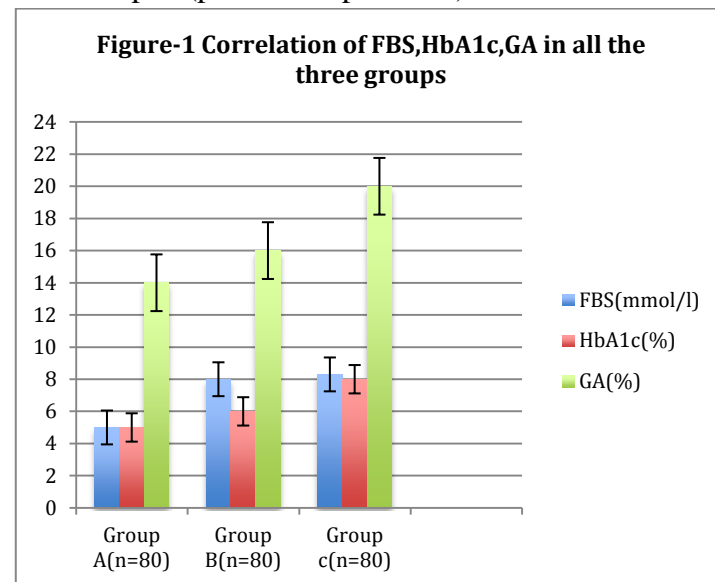
SD: standard deviation, P < 0.001; Highly significant

In the present study the mean serum ADA levels in Group A were 17.45 ± 7.25 U/L, in Group B were 31.04 ± 9.25 U/L whereas in Group C were 46.23 ± 8.25 U/L. Statistical analysis showed that the mean serum ADA levels of Group C were significantly higher than Group B (p<0.001) and the levels of ADA were significantly higher in both Group B and Group C as compared to Group A (p<0.001) [Table-3].The mean serum uric acid levels in Group A were 6.25 ± 1.75 mg/ dl, in Group B were 7.10 ± 1.90 mg/dl and in Group C were 5.45 ± 1.55 mg/dl. The mean serum uric acid levels of Group B were significantly higher than Group C (p<0.001) whereas levels of mean serum uric acid in Group C were significantly lower than Group A (p=0.018) but no significant difference was observed between Group A and Group B (p=0.290) Table.3:

Figure-2 Comparison of ADA and serum uric acid levels in all the three groups
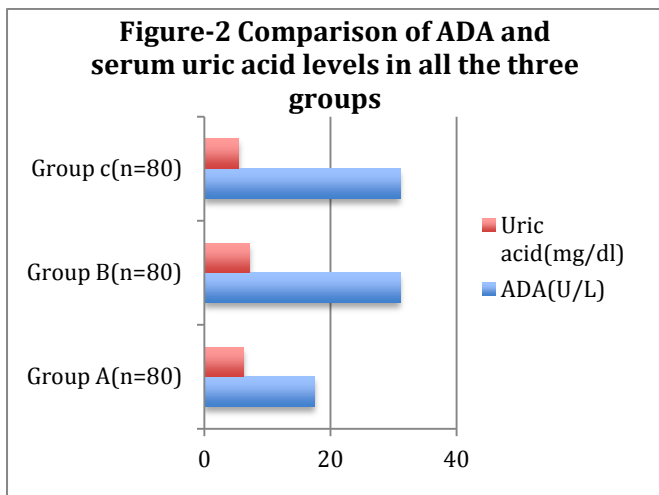
SD: standard deviation, P >0.05; not significant; *P<0.05 significant at 5% significant level ***P < 0.001; Highly significant.

The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and GA, levels in Group B showed positive correlation between GA(r=0.005),HbA1$_c$ (r=0.004) and ADA. Similarly when the comparison was made between serum uric acid levels with GA(r=0.198) there was positive correlation. [Figure-3]. The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and GA, levels in Group C showed positive correlation between GA(r=0.138), and ADA. When the comparison was made between serum uric acid levels with GA(r= -0.016) and HbA1$_c$(r= -0.018).there was negative correlation.[Figure-3]

Figure-3 Comparison of serum ADA, uric acid levels with GA levels in all the three groups.
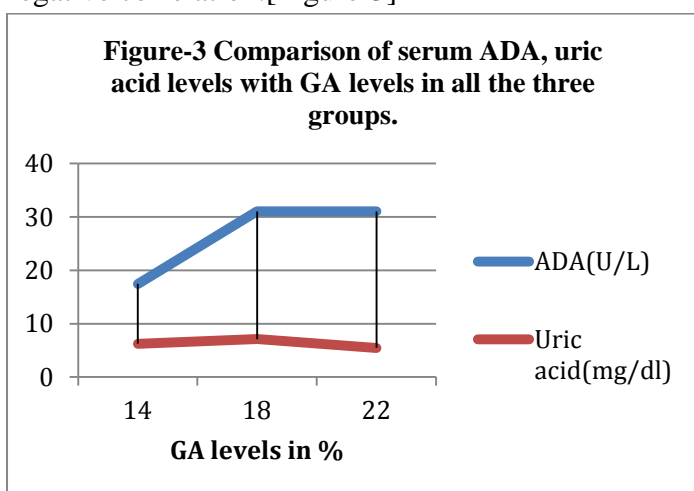
r value: Pearson correlation coefficient; *correlation is significant at the 0.05 level; **correlation is significant at the 0.05 level.

Discussion

Type 2 Diabetes is a heterogeneous disorder characterized by improper carbohydrate, fat and protein metabolism because of developed insulin resistance[14]. .As type 2 diabetes mellitus progresses its complications are severe and becomes epidemic which leads to various consequences[15]. And It is characterized by relative deficiency of insulin or developed insulin resistance. In the present study, we observed that the mean serum ADA levels of Group C were significantly higher than Group B (p< 0.001). Also the levels of ADA were significantly higher in both Group B and Group C than Group A (p < 0.001).these results were similar to the other studies reported by Hoshino T et al[5] and Kurtal N et al[16]. Advanced glycation End Products (AGEs) are formed because of prolonged hyperglycemia and results non-enzymatic reactions between intra-cellular glucose-derived dicarbonyl precursors with the amino group of both intracellular and extracellular proteins[17]. These advanced glycation end products stimulate receptors (RAGE) and their interaction is might be the reason to increase the complications of diabetes[18]. If we identify the insulin resistance at early stage it will be very helpful in minimizing the complications of type 2 diabates. Insulin resistance can be identified by estimating the ADA levels which is a simple, inexpensive marker without actually requiring estimation of serum insulin required in current methods for measuring insulin resistance. All subjects were interviewed for details of their age, weight, height, and smoking, Alcoholism. And also, blood pressure and body mass index (BMI) were measured for each subject. This increase was more than two times in group B and group C than in group A Table-1. There is a positive significant correlation between GA, HbA1c and FBS as shown in Figure 1. Our results shows, that adenosine deaminase (ADA) activities were significantly increased in both the diabetic groups (group B & C) along with uric acid levels as compared to control group Figure 2. The

Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and GA levels in Group B showed positive correlation between GA and ADA (r=0.005) (r=0.004) but the difference was not statistically significant (p=0.993) which means that with the increase in GA levels, serum ADA were also increased. These findings were in accordance with the study of Kurtul N et al.[16]. Similarly when the comparison was made between GA levels and serum uric acid, there was positive correlation (r=0.198) which were statistically not significant (p=0.300). The increased activity of ADA activity might be the reason for increased uric acid levels in this group and also High influx of glucose 6-phosphate through hexose monophosphate shunt might be another reason as a result of impaired glycolytic pathway. This findings were in accordance with study by Dehghan A et al[19] and Modan M et al.[20]. The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and GAlevels in Group C showed positive correlation between GA and ADA (r=0.138). Although statistically not significant (p=0.542). When the comparison was made between serum uric acid levels and GA there was negative correlation (r=-0.016) which were statistically not significant (p=0.958) meaning thereby that when the levels of GA increased more than 6%,there is decrease in uric acid levels. The reason for this finding is thought to be due to the uricosuric effect of glycosuria.

Conclusion

In conclusion our study suggests that there is an increase in serum ADA levels with increase in GA levels, which may play an important role in determining the glycemic status in diabetes. Furthermore, it was found that the serum uric acid levels increased with moderately increasing levels of GA (<20%) and then decreased with further increasing levels of GA(>20%). Serum ADA and serum uric acid levels reflect closely related components of the same disease i.e. Type 2 Diabetes Mellitus.

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