Fasting Plasma Total Amino Acid Levels in Relation to Glucose Levels in Type 2 Diabetes Mellitus – A Study from Karnataka, India

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ABSTRACT

BACKGROUND

Hyperglycemia, observed in type 2 diabetes mellitus (T2DM) patients, is the primary root cause for the development of life threatening - diabetes induced micro- & macro-vascular complications. Generally, in fasting state, gluconeogenesis accounts for about 30 % of overall hepatic glucose output and the increase in type 2 diabetic subjects may be much more than this level. The majority of gluconeogenic precursors may originate from muscle glycolysis and in the form of glucogenic amino acids including alanine. We wanted to assess fasting plasma total amino acid levels in T2DM patients and correlate the levels with the glycaemic status of T2DM patients in this study.

METHODS

The observational comparative study was undertaken at Research and Development department, from October 2019 to November 2020 and it consisted of 157 subjects, including 75 normal control subjects, and 82 T2DM patients. The T2 diabetic patients attending medical OPD of Subbaiah Institute of Medical Sciences, Purle, Shivamogga (SUIMS) were randomly selected and were sub-grouped in to 3 groups depending on their fasting plasma glucose (FPG) levels. The group 1 patients were having FPG level in the range of 150 – 200 mg / dl, group 2 patients having in the range of 201 - 250 mg / dl and the group 3 patients were having FPG above 251 mg / dl.

RESULTS

It is evident from the present study that FPG levels as well as AAN levels are significantly ($P > 0.001$) elevated in T2DM patients as compared to normal control subjects and there is a proportional rise in AAN levels in relation to FPG Levels in T2dm patients.

CONCLUSIONS

The rise in plasma AN levels is proportional to glycemic status in T2DM patients and can be employed to assess the glycemic status in these patients.

KEY WORDS

Amino Acids, Gluconeogenesis, Type 2 Diabetes Mellitus

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BACKGROUND

Hyperglycemia, observed in type 2 diabetes mellitus (T2DM) patients, is the root-cause for the development of life threatening – diabetes induced micro- & macro-vascular complications,^{1,2} like retinopathy, nephropathy, cardiopathy and neuropathy. A prompt regulation of blood glucose level is very much essential to abolish or to delay the onset of these complications. One of the prominent endogenous contributor of blood glucose is gluconeogenesis, the process of formation of glucose from non-carbohydrate metabolites which is generally increased in diabetes mellitus mainly due to deficiency of insulin - sole hypoglycemic hormone, as insulin is a known gluconeogenic suppressor.³ Among the various gluconeogenic metabolites glucogenic amino acids are significant.

Insulin, apart from facilitating the glucose transport across the cell membranes as well as increasing glucose utilisation by cells, is also known to suppress tissue proteolysis,⁴⁻⁶ a prime source of free amino acids which may be utilized for gluconeogenesis. The deficiency of insulin as observed in T2DM patients may cause unsuppressed proteolysis which provides significant amount of glucogenic amino acids hence facilitating increased formation of glucose through gluconeogenesis. The rise in plasma amino acid levels has been observed by many researchers⁷⁻¹¹ and the rise specifically in branched - chain amino acid levels has been noted by few earlier workers.¹²⁻¹⁴ whereas few others have observed a significant fall in serum total amino acids in T2DM patients.^{15,16}

Objectives

1. To estimate fasting plasma glucose and total amino acid levels in T2DM patients.
2. To correlate total amino acids levels with the glycaemic status of T2DM patients.

METHODS

The observational comparative study, involving the T2DM patients attending medical outpatient department (OPD) of Subbaiah Institute of Medical Sciences (SUIMS), Shivamogga and its affiliated hospitals, in the age group of 30 - 55 years were randomly selected. A detailed history regarding the illness was collected from these patients. Normal control subjects in the age group of 30 - 55 years were taken from the employees of SUIMS and its affiliated hospitals, Shivamogga.

Diabetic patients below the age of 30 years and above 55 years, those with psychiatric disorders as well as the patients receiving hormone therapy were excluded from the study.

The present work was undertaken at Research and development department, SUIMS from October 2019 to November 2020 and the study consisted of 157 subjects, including 75 normal control subjects and 82 T2DM patients. These details are narrated in Chart 1.

A fasting heparanized blood sample (5 - 6 ml) was collected from both T2DM patients and from normal control subjects after obtaining a written informed consent from each one of them. The samples were centrifuged at 3000 rpm for 6 - 8 min and the separated plasma was employed for the estimation of FPG¹⁷ and plasma total amino acid nitrogen

(AAN)¹⁸ levels.

Glucose Estimation

Plasma glucose was estimated by GOD - POD procedure.¹⁷ The hydrogen peroxide liberated upon oxidation of glucose by glucose oxidase was acted upon in site by peroxidase and the produced nascent oxygen converted anisidine to give quantitatively a coloured complex which was measured photometrically.

AAN Estimation

The amino acids present in tungstate protein free filtrate of plasma were determined by Folin method.¹⁸ using 1 - Amino, - 2 - naphthalo - 4 - sulphonic acid and the greenish coloured complex produced was measured photometrically at the type 2 diabetic patients who were sub-grouped in to 3 groups depending on their fasting plasma glucose (FPG) levels. The group 1 patients were having FPG levels in the range of 150 - 200 mg / dl, group 2 patients in the range of 201 - 250 mg / dl and the group 3 patients were having FPG above 251 mg / dl.

Statistical Analysis

The data obtained was statistically analyzed using SPSS version 16 software. Student's t-test was used to ascertain the significance and the P value less than 0.05 was considered statistically significant.

RESULTS

Group	FPG mg / dl
Group1 (26)	150 - 200
Group 2 (28)	201 - 250
Group 3 (28)	< 251

Table 1. Distribution of T2DM Patients According to Their FPG Levels

Note - The number in parentheses indicates the number of patients.

	FPG mg / dl	AAN
Normal Controls (75)	90.15 ± 13.80	6.75 ± 1.66
T2DM Patients (82)	207.95*** ± 36.80	16.52*** ± 4.72

Table 2. Plasma Levels of Fasting Glucose (FPG), and Total Amino Acid Nitrogen (AAN), in Normal Control Subjects as Well as in T2DM Patients

Note

1. The values are expressed as their Mean ± SD.
2. The number in parentheses indicates the number of subjects.
3. Probability * P > 0.05, **P > 0.01 and ***P > 0.001.

T2 DM Sub - Groups	FPG Mg/dl	AAN
Group 1 (FPG - 150 - 200 mg / dl) (26)	181.14 ± 22.50	8.55 ± 1.20
Group 2 (FPG - 201 - 250 mg / dl) (28)	228.60** ± 18.80	11.54*** ± 1.80
Group 3 (FPG - Above 251 mg / dl) (28)	260.72** ± 28.80	12.14** ± 1.75

Table 3. Plasma Levels of FPG, and AAN, in T2DM Patients Sub-Groups (Group 1, Group 2 and Group 3)

Note

1. The values are expressed as their Mean ± SD.
2. The number in parentheses indicates the number of subjects.
3. Probability * P > 0.05, **P > 0.01 and ***P > 0.001.

The results obtained in the present study are depicted in Table 1 and Table 2. Table1 shows the level of FPG and AAN in normal control subjects as well as in T2DM patients. It is

evident from the table that FPG levels as well as AAN levels are significantly ($P > 0.001$) elevated in T2DM patients as compared to normal control subjects suggesting a close relationship between FPG levels and AAN levels in these T2DM patients. Table 2 depicts AAN level in group 1, group 2 and in group 3 T2DM patients. It is seen from the table that there is a proportionate rise in AAN levels in relation to FPG levels suggesting that amino acids maybe the principle contributors

for glucose rise in these diabetic patients. Figure 1a, 1b and 1c shows the comparative variation graphs of TAA and FPG levels in Group 1, Group 2 and Group 3 T2DM patients. It is clear from the graphs that there is a direct relationship between FPG levels and plasma TAA levels suggesting that TAA levels may be related to the glycemic condition of a T2DM patient as well as an indicator of glycemic status in these patients.

DISCUSSION

The circulating amino acids, which are related to insulin action through their tissue proteolysis suppression effect, may

serve as novel biomarkers for predicting the risk of diabetic complications that may enlighten hitherto unknown pathways of diabetic pathophysiology^{7,19,20} as well as may explain the link between amino acids and the

gluconeogenesis. Insulin, the lone hypoglycaemic hormone is known to influence many cellular metabolic reactions including gluconeogenesis, tissue proteolysis and tissue lipolysis through regulating the key enzymes involved in these pathways. The chart shown in Figure 2 clearly explains many significant actions of insulin, gluconeogenesis being the most significant among them.

Generally, in fasting state, gluconeogenesis accounts for about 30 % of overall hepatic glucose output.¹⁹⁻²⁴ and the increase in type 2 diabetic subjects may be much more than this level.^{25,26} The majority of gluconeogenic precursors may originate from muscle glycolysis and in the form of glucogenic amino acids including alanine.²⁴ Apart from pyruvate and lactate, the amino acids are the significant gluconeogenic precursors and contribute to blood glucose levels in fasting as well as in diabetic state. It has been observed in our earlier studies that in alloxan diabetic rats the plasma as well as the liver tissue amino acids were significantly elevated which may be due to sub normal functions of insulin as insulin has a suppression effect on tissue proteolysis. This unsuppressed proteolysis in diabetic state supplies amino acids for endogenous glucose formation which is again favoured by subnormal insulin action as hormone insulin is the lone suppressor of the process gluconeogenesis.

In the present study it has been observed that there is a significant rise in plasma AAN levels in type 2 diabetic subjects which is in line with the observation made by earlier workers,²⁷⁻²⁹ suggesting a close relationship between glucose metabolism and amino acid metabolism which may be established through transamination reactions.

The key amino acid keto acid pairs involved in transamination reactions are glutamate- α -ketoglutarate, aspartate-oxaloacetate and pyruvate - alanine. Insulin, not only decreases the formation of glucose through gluconeogenesis by suppressing the activities of key gluconeogenic enzymes but also suppresses the tissue proteolysis⁴⁻⁶ thereby decreasing the availability of amino acids for gluconeogenesis. Among the various glucogenic metabolites, glucogenic amino acids specifically alanine, aspartate and glutamate are significant.

Altered branched chain amino acid and aromatic amino acid levels have been reported in T2DM patients which may be due to an increased proteolysis because of sub normal insulin action. The glucogenic amino acids specifically alanine, aspartate and glutamate, derived from rised proteolysis maybe utilized as glucogenic precursors and the rest of the amino acids, branched chain and aromatic amino acids may show a rise in T2DM patients, hence the rise observed in the present study in plasma AAN substantiate the earlier findings.^{11,13,15,16,27}

The glycemic status regulation, which is partly dependent on the hepatic glucose output through gluconeogenesis, is significant in T2DM patients in delaying the onset of diabetic complications.

It is evident from the table 2 and the graphs in Fig 1 that the glycemic status in group 1, group 2 and group 3 T2DM patients is proportional to plasma total amino acid levels suggesting a close relationship between glycemic status and plasma total amino acids in these patients (refer table 2 & Figure 1).

Further it has been shown by earlier workers that plasma total amino acids may predict the diabetic complications.^{12,14,29} resulting from uncontrolled hyperglycemia.

The present study establishes the fact that plasma total amino acids are significantly elevated in T2DM patients and this elevation is proportional to the glycemic status in these patients. Further the study establishes the need for development of agents which substantially lowers the tissue photolytic rate, the process which provide amino acids for gluconeogenesis, hence may result in regulation of glycemic status.

CONCLUSIONS

A significant rise in plasma AAN seen in T2DM patients may substantially contribute to the glycemic status of T2DM patients and may serve as an index to the gluconeogenic rate in these patients. The study also suggests a proper medication to be administered towards controlling or minimizing tissue proteolysis caused by insufficient action of insulin in these patients that may facilitate in achieving a prompt required glycaemic control.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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