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Plasma Total Amino Acids, Plasma Glutamate & Alanine Levels in Diabetic Subjects

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Abstract- Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output, the increase in type 2 diabetic subjects may be much more than this level. There is a close relationship between glucose metabolism and amino acid metabolism which is established through transamination reactions. The key amino acid -keto acid pairs involved in transamination reactions are glutamate – α ketoglutarate, aspartate – oxaloacetate and pyruvate - alanine. A study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects. The separated plasma samples were employed for the estimation of total amino acid nitrogen levels, as well as for the estimation of plasma alanine and glutamate levels. The results indicate a significant rise in the plasma levels of total amino acids, alanine & glutamate in type-2 diabetic subjects as well as a significant increase in plasma alanine levels in diabetics of 30- 40 yrs age as compared to diabetics of 41-60yrs of age. These findings suggest an increased availability of glucogenic amino acid precursors for glucose formation may be due to lack of proteolytic suppression of insulin.

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Abstract- Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output, the increase in type 2 diabetic subjects may be much more than this level. There is a close relationship between glucose metabolism and amino acid metabolism which is established through transamination reactions. The key amino acid -keto acid pairs involved in transamination reactions are glutamate – α ketoglutarate, aspartate – oxaloacetate and pyruvate - alanine. A study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects. The separated plasma samples were employed for the estimation of total amino acid nitrogen levels, as well as for the estimation of plasma alanine and glutamate levels. The results indicate a significant rise in the plasma levels of total amino acids, alanine & glutamate in type-2 diabetic subjects as well as a significant increase in plasma alanine levels in diabetics of 30-40 yrs age as compared to diabetics of 41-60yrs of age. These findings suggest an increased availability of glucogenic amino acid precursors for glucose formation may be due to lack of proteolytic suppression of insulin.

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I. INTRODUCTION

Glucose, the process of formation of glucose from non-Carbohydrate metabolites is generally increased in Diabetes mellitus mainly due to deficiency of insulin as insulin has a gluconeogenic suppression effect (13). Among the various Glucogenic metabolites, glucogenic amino acids alanine, aspartate and glutamate are significant. The transamination of these amino acids through respective transaminases yield the ketoacids: α -ketoglutarate, oxaloacetate and pyruvate, which are readily convertible to glucose through gluconeogenesis. Insulin apart from its hypoglycemic action also has a

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tissue proteolysis suppression effect thereby decreasing the availability of amino acids. Hence a deficiency of insulin as observed in diabetes mellitus may lead to increased gluconeogenesis due to the lack of suppression effect of gluconeogenesis on one hand and the lack of suppression effect of tissue proteolysis on the other hand. There are no much studies available regarding the plasma levels of glucogenic amino acids in particular glutamate and alanine levels in diabetic subjects. Garcia et al reported that increased glucose recovery was observed in insulin induced hyperglycemia in rats upon oral or intraperitoneal glutamine or alanine administration to these rats (14) suggesting a close relationship between plasma amino acid and plasma glucose levels. The reports of Agustino consoli et al suggest that increased conversion of alanine to glucose in NIDDM subjects. Further it has been proposed that increased muscle glycolysis might provide additional alanine and lactate to sustain gluconeogenesis in type 2 diabetic subjects (1).

Hence a study was undertaken to assess the plasma levels of total amino acids, glutamate and alanine in type 2 diabetic subjects.

II. MATERIALS & METHODS

The type 2 diabetic subjects in the age group of 30-60 years visiting the medical OPD of SUBBAIAH INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTER, PURLE, SHIMOGA, were randomly selected. Age matched normal subjects were randomly selected from the employees of the college and hospital. A blood sample (5ml) with heparin as an anticoagulant was collected in the fasting state, from each of the selected normal as well as diabetic subjects after obtaining an informed consent from them. The blood samples were centrifuged at 3600rpm for 8 minutes to separate plasma. The separated plasma samples were employed for the estimation of total amino acid nitrogen (AAN) levels (9), as well as for the estimation of plasma alanine (AL) and glutamate (GM) levels using quantitative paper chromatographic procedure (18). Ethical clearance from the institutional research council, for the present work was taken.

III. RESULTS

The study included 80 normal subjects of both sexes in the age group of 30-50yrs and 103 type 2 diabetic subjects of both sexes in the age group of 30-

60yrs. The normal subjects included equal numbers of male and female subjects. The type 2 diabetic subjects included 85 male diabetics and 38 female diabetics. Among the diabetic subjects 31 were with family history of diabetes. A split in the diabetics as per the age, there were 36 in the age group of 30-40yrs, 67 in the age group of 41-60yrs. These groupings or division of the normal subjects and diabetic subjects are given in chart 1.

Table-1 shows the levels of total amino acid nitrogen (AAN) alanine (AL) and glutamate (GM) levels in plasma in normal and type 2 diabetic subjects. It is seen from the table that the plasma levels of AAN, AL and GM are significantly elevated in type 2 diabetic subjects as compared to normal subjects may be due to lack of insulin.

Table 2 and table 3 narrate the plasma levels of AAN, AL and GM in diabetic male subjects and diabetic female subjects as compared to their normal counter parts respectively. It is evident from the tables that in both male as well as in female diabetics the plasma levels of AAN, AL and GM are significantly raised as compared to their normal counter parts showing that the diabetics induced alteration in these parameters is common to both sexes.

Table 4 gives the plasma levels of AAN, AL and GM in male diabetic subjects as compared to female diabetic subjects. It is seen from the table that there is a significant rise in GM levels in female diabetics as compared to male diabetic subjects where as there is no significant change observed in AAN and AL levels among these two groups.

Table 5 gives the plasma levels of AAN, AL & GM in diabetic subjects of different age groups i.e. diabetics in the age group of 30-40yrs and diabetics in the age group of 41-60yrs age group. It is evident from the table that a significant rise in plasma AL in diabetics of 30-40 yrs age as compared to diabetics of 41-60yrs of age. Whereas no significant changes observed between these two groups in plasma AAN and plasma GM levels

IV. DISCUSSION

Generally, in fasting state, gluconeogenesis accounts for about 30% of overall hepatic glucose output (1, 6), the increase in type 2 diabetic subjects may be much more than this level (2-6, 8, 12). The majority of gluconeogenic precursors may originate from muscle glycolysis and in the form of alanine (7). In the present study it has been observed that there is a significant rise in plasma AL and GM levels in type 2 diabetic subjects which is in line with the observation made by Augustino Consoli et al (1). There is a close relationship between glucose metabolism and amino acid metabolism which is 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, the sole hypoglycemic hormone, not only decreases the formation of glucose through gluconeogenesis by suppressing the activities of key gluconeogenic enzymes but also suppresses the tissue proteolysis (11, 15, 17) thereby decreasing the availability of amino acids for gluconeogenesis. The results of present study with type 2 diabetic subjects clearly establishes an elevated plasma levels of AAN, AL, and GM in type 2 diabetic subjects (refer table 1) which may be due to decreased insulin levels or due to decreased insulin action. This decreased insulin levels in these subjects might have caused a rise in the plasma levels of AAN, AL and GM as insulin is known to possess a suppression effect on tissue proteolysis (13). A parallel increase in plasma GM and AL levels along with AAN levels in type 2 diabetic subjects as compared to their normal counter parts (ref table 1, 2 and 3) suggests that the increase in plasma AAN in these subjects may be partly due to an increase in these two amino acids (plasma AL and GM levels). The increase in plasma and probably tissue GM levels in type 2 diabetic subjects (male and female diabetic subjects), as compared to their normal counterparts may further suppresses the utilization of glucose adding to hyperglycemia as it is shown by Visweswaran and Subramanyan (20) that added glutamic acid could reduce the glucose utilization.

Further a little elevation in the plasma levels of AAN, AL and GM observed in the present study in female diabetic subjects as compared to male diabetic subjects which may be due to hormone prolactin which has a diabetogenic effect (10) and to hormone estrogen, which acts through releasing biogenic amines (19).

When the plasma levels of AAN, GM and AL in diabetic subjects of different age group (diabetes of 30-40 yrs and diabetic of 41-60yrs age group are compared a significant rise is seen in the present studies in plasma AL levels in diabetics of 30-40yrs age group as compared to diabetics of 41-60yrs age group, which may probably be due to decreased glucose utilization with increasing age as well as due to increased tissue response to decreased insulin levels as there may be an increased tissue proteolysis in the early stages of diabetic disease, as much tissue wastage and weight loss is seen in early stage of diabetic disease. It can be concluded by the present studies that there is a significant rise in plasma levels of AL and GM in type 2 diabetic subjects of both sexes which may be due to increased proteolysis because of insulin deficiency, also a significant rise observed in plasma AL level in diabetics of 30-40yrs age group as compared to other groups.

CHART—1

Chart showing the division of normal and diabetic subjects according to different parameters.

Total number of subjects in the present study	----183
Total type 2 diabetic subjects	---- 103
Total normal subjects	-----80
Normal male subjects	---- 40
Normal female subjects	----40
Male diabetic subjects	-----65
Female diabetic subjects	-----38
Diabetic with family history	-----31
Diabetic without family history	----67
Diabetic subjects in the age group of 30--40yrs	----36
41—60yrs	----67

Table 1 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in whole Blood of Normal and Diabetic Subjects

Parameter	Normal Subjects (n=80)	Diabetic Subjects (N=103)
Total Amino acid nitrogen (mg/dl)	6.26 ± 0.71	17.55*** ± 4.43
Glutamic acid (mg/dl)	3.75 ± 0.68	9.86*** ± 2.43
Alanine (mg/dl)	2.95 ± 0.53	5.55*** ± 1.40

Note: 1. The number in parenthesis shows the number of samples.
 2. Values are expressed as their Mean + SD
 3. p- value *p<0.05, **p<0.01, ***p<0.001

Table 2 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Normal Male and Diabetic Male Subjects

Parameter	Normal Male Subjects (n=40)	Diabetic Male Subjects (N=65)
Total Amino acid nitrogen (mg/dl)	8.26 ± 0.72	15.17*** ± 4.38
Glutamic acid (mg/dl)	3.60 ± 0.72	8.92*** ± 2.38
Alanine (mg/dl)	2.94 ± 0.51	5.30** ± 1.33

Note: 1. The number in parenthesis shows the number of samples.
 2. Values are expressed as their Mean + SD
 3. p- value *p<0.05, **p<0.01, ***p<0.001



Table 3 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Normal Females and Diabetic Females Subjects

Parameter	Normal Female Subjects (n=40)	Diabetic Female Subjects (N=38)
Total Amino acid nitrogen (mg/dl)	8.60 ± 0.71	17.70*** ± 3.92
Glutamic acid (mg/dl)	3.80 ± 0.72	11.53*** ± 2.61
Alanine (mg/dl)	3.12 ± 0.54	6.24** ± 1.47

Note: 1. The number in parenthesis shows the number of samples.
 2. Values are expressed as their Mean + SD
 3. p- value *p<0.05, **p<0.01, ***p<0.001

Table 4 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Blood of Diabetic Male and Diabetic Female Subjects

Parameter	Diabetic Male Subjects (n=40)	Diabetic Female Subjects (N=65)
Total Amino acid nitrogen (mg/dl)	15.17 ± 4.38	17.70 ± 3.92
Glutamic acid (mg/dl)	8.92 ± 2.38	11.53 ± 2.61
Alanine (mg/dl)	5.30 ± 1.33	6.24 ± 1.47

Note: 1. The number in parenthesis shows the number of samples.
 2. Values are expressed as their Mean + SD
 3. p- value *p<0.05, **p<0.01, ***p<0.001

Table 5 : Table showing the levels of Total Amino acid nitrogen, Glutamic acid and Alanine in Whole Blood Diabetic Subjects with different age-group

Parameter Age Group	Total Amino acid nitrogen (mg/dl)	Glutamic acid (mg/dl)	Alanine (mg/dl)
30-40 years (N=49)	15.75 ± 4.11	9.6 ± 2.09	8.44** ± 2.09
41-60 years (N=35)	15.04 ± 2.60	8.68 ± 0.30	4.03 ± 0.49

Note: 1. The number in parenthesis shows the number of samples.
 2. Values are expressed as their Mean + SD
 3. p- value *p<0.05, **p<0.01, ***p<0.001

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