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Semi quantitative estimation of amino acids excreted in urine of diabetic patients

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Abstract

Diagnosis of Diabetes in its very early stage will become very important in the days to come to prevent the morbidity and mortality of the patients. From this study a relationship between amino acid losses in urine of diabetic patients and their glycaemic control has been established. The observations of this study indicate that analysis of acidic, branched chain, aromatic amino acids and in particular, the measurement of His, Glu, Met, in the urine of diabetic patients under treatment holds significance, as it can be used as an index of hyperglycaemia and effectiveness of treatment. Since urine samples can be easily obtained from patients and Chromatographic method of amino acid estimation is cheap and convenient, it can be easily used for semi quantitative estimation of certain amino acids.

Keywords: amino acids, diabetes mellitus, chromatography

Introduction

Various studies have established generalized aminoaciduria occurs in all types of Diabetes, there is relative paucity of information regarding the amount of amino acids excreted by Diabetic patients under treatment and degree of aminoaciduria with control of Diabetes. Hence the following study is undertaken to identify the amino acids in the urine of all types of Diabetic patients under treatment as well as normal individuals and an approximate estimation of five amino acids in Diabetic urine: Met (sulphur containing), His (basic), Phe (aromatic), Ile (branched chain) & Glu (dicarboxylic) with the help of Paper Chromatography. The magnitude of increase in protein breakdown is more than protein synthesis during Insulin deprivation; so there is net protein loss in Insulin deprivation. Regional studies have shown that in skeletal muscle there is net increase in protein breakdown during Insulin deprivation, resulting in net release of amino acids^[18].

Generalized aminoaciduria is a direct effect of glucose on amino acid reabsorption and not a specific alteration of transporter transcription. The most likely explanation is that the presence of glucose in proximal renal tubule depolarizes and dissipates the electrical gradient of Sodium dependant amino acid transporters, causing a reduction in amino acid reabsorption^[2].

Aims and Objectives

Semi-quantitative estimation of five amino acids: Methionine (sulphur containing), Histidine (basic), Isoleucine (branched chain), Phenylalanine (aromatic) and Glutamic acid (acidic) in urine samples of Diabetic patients as well as normal individuals by the method of Chromatography.

Materials & Methods

Subjects

A total 30 patients of both sexes suffering from either Type 1 or Type 2 Diabetes Mellitus and 30 age and sex matched normal control subjects were selected for the study. Pregnant individuals, patients having any concomitant disease that can alter urinary excretion of amino acids like various types of aminoaciduria and patients with established Diabetic nephropathy were excluded from the study.

Samples

For Chromatography: Early morning urine samples were collected from all participants. The urine samples were centrifuged to remove any cells or casts, filtered and then the filtrate was heated with Sulphosalicylic acid.

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For Blood Glucose estimation and estimation of Serum creatinine: 2ml of fasting venous Blood samples were collected.

Materials Used

For Chromatography: Chromatography chamber, Whatman Cellulose Chromatography Papers Grade No.1, size: 46x57cm, Chr...3001-917, thickness 0.18mm, Butanol, Glacial Acetic acid, Amino acids standard kit, Ninhydrin GR, Sprayer, Hot air blower.

Methods

Semi-quantitative estimation of amino acids in urine

Standardization: Separate solutions of five amino acids: His, Ile, Met, Phe & Glu of known concentration⁷ were prepared: Met (15µmol/dl), His (1000µmol/dl), Ile (8µmol/dl), Phe (60 µmol/dl), Glu (70 µmol/dl). Serial dilutions of each of these amino acids in concentration 1, ½, ¼, 1/8, 1/16 parts were performed resulting in five solutions respectively for each

amino acid. A series of 5 spots of serial dilutions of each amino acid solution were applied on a separate paper as 4µl spots 2.5cm from the paper edge. These papers were then allowed to develop as above which formed the standard colour paper for that respective amino acid. The areas of the spots of serial dilutions of each amino acid were measured by square counting on graph paper. A Standard graph is plotted for each of the six amino acids with concentration of the amino acid taken on Y-axis and spot area on X-axis. Semi-quantitative estimation From the above chromatograms obtained during detection of amino acids in urine samples of both NI and DP, the area of the spots which were identified as one of the five amino acids i.e. Met, His, Ile, Phe & Glu were measured by square counting (in sq cm) on graph paper. These spot areas were then compared with the spot areas on the standard chromatograms of the respective amino acids and hence an approximate concentration of these six amino acids (in µmol/dl) in the urine samples was obtained.

Result

Observations

Table 1: Approximate Concentration of 5 Amino Acids in Urine of Normal Individuals (µmol/dl)

NI	Met	His	Phe	Ile	Glu
1	0.94	2.34	nd	0.5	nd
2	nd	nd	3.75	Nd	8.75
3	nd	3.12	nd	Nd	17.5
4	nd	nd	15	Nd	35
5	nd	3.12	nd	0.5	17.5
6	nd	6.25	nd	0.5	10.93
7	0.94	3.12	0.94	0.25	35
8	3.75	12.5	nd	0.125	35
9	1.88	nd	nd	0.1	17.5
10	1.41	6.25	nd	0.125	17.5
11	nd	nd	nd	0.1	2.92
12	0.94	nd	nd	.25	10.93
13	0.47	nd	nd	Nd	1.46
14	0.94	3.12	nd	0.75	2.92
15	0.47	nd	nd	Nd	2.92
16	1.88	nd	nd	.25	35
17	1.88	nd	nd	Nd	35
18	0.94	nd	nd	.25	17.5
19	0.47	nd	nd	0.125	nd
20	1.88	6.25	nd	Nd	20.41
21	0.47	nd	3.75	Nd	8.75
22	0.235	nd	nd	Nd	nd
23	1.41	1.56	nd	1	10.93
24	0.47	nd	nd	Nd	4.38
25	3.75	1.56	nd	0.125	8.75
26	nd	nd	nd	Nd	8.75
27	1.41	nd	3.75	1	17.5
28	0.94	nd	nd	.25	1.46
29	nd	nd	nd	Nd	1.46
30	nd	nd	nd	Nd	0.73

Table 2: Approximate Concentration of 5 Amino Acids in Urine of Diabetic Patients In (µmol/dl)

DI	Met	His	Phe	Ile	Glu
1	4.28	nd	nd	2	2.92
2	nd	6.25	nd	1	35
3	nd	12.5	0.94	0.75	17.5
4	6.42	25	5.62	4	70
5	nd	6.25	1.87	nd	35
6	1.41	12	3.75	1.5	58.33
7	0.94	12.5	nd	0.5	35
8	nd	nd	nd	0.25	8.75

9	4.28	20	7.5	7.5	93.33
10	6.42	12.5	nd	2	70
11	3.75	20	7.5	7.5	93.33
12	3.12	20	3.75	8	93.33
13	1.88	12	187	7.5	70
14	0.94	6.25	nd	1	35
15	3.75	nd	5.62	2	93.33
16	nd	nd	nd	1	35
17	nd	6.25	60	1.5	35
18	6.42	20	3.75	0.25	70
19	3.75	20	3.75	4	81.66
20	0.47	6.25	0.94	2	35
21	6.42	nd	nd	4	93.33
22	nd	3.12	30	2	35
23	nd	1.56	1.87	2	17.5
24	nd	12.5	7.5	0.75	58.33
25	0.94	12.5	3.75	3	93.33
26	0.47	3.12	nd	1	35
27	6.42	6.25	nd	0.5	81.66
28	nd	4.66	15	0.5	35
29	0.47	3.12	nd	nd	17.5
30	nd	6.25	3.75	5	70

Statistical Analysis

Comparison of concentration of particular amino acid present in normal individuals and diabetic patients

“Z-Test for equality of two means” was performed in order to study whether there is a difference in concentration of particular amino acid (Met., His., Phe, Glu, Ile) present in normal individuals and diabetic patients. Here the value of z statistic for all the test of all the five amino acids were calculated separately at both 5% ($z \leq -1.64$) and 1% ($z \leq -2.33$) level of significance by the formula:

$$Z = \frac{\text{Mean of Sample 1} - \text{Mean of Sample 2}}{\sqrt{[(\text{variance of Sample 1}/n_1) + (\text{Variance of Sample 2}/n_2)]}}$$

Table 3

Amino acid	Details
Methionine	Calculated value of Z - 2.4643 \leq - 2.33
Histidine	Calculated value of Z - 5.1980 \leq - 2.33
Phenylalanine	Calculated value of Z - 2.1465 \leq -1.64 but Z -2.1465 $>$ -2.33
Isoleucine	Calculated value of Z -0.060 $>$ -1.64
Glutamic acid	Calculated value of Z - 7.2128 \leq -2.33

From the table we find that for Met, His & Glu, $z \leq -2.33$; therefore we accept the hypothesis “Mean concentration of these individual amino acids is greater in Diabetic patients than normal individuals” while for Phe & Ile, $z \geq -2.33$; therefore we reject the above hypothesis for these 2 amino acids. Hence we conclude that Concentration of Met, His & Glu is more in diabetic patients than normal individuals, while there is no significant difference in the concentration of Phe & His between diabetic patients and normal individuals.

Discussion

This study is about semi-quantitative estimation of 5 amino acids chosen one from each group: Glutamic acid (dicarboxylic), Histidine (dibasic), Methionine (sulphur containing), Phenylalanine (aromatic) and Isoleucine (branched chain). It was found that Glu was excreted in all Diabetic patients (100%), followed by Aspartic acid (93.33%) both of them being dicarboxylic acid. Other amino acids like His (83.33%), Pro (80%), Met and Gly (73.33% each) Ile

(70%), Phe (63.33%) were found in considerable amount of patients.

Degree of aminoaciduria with control of Diabetes depicted was also studied. A positive co-relation of degree of aminoaciduria was found with their HbA1c values. (R=0.89). Hence it can be said that patients with poorer control of Diabetes by Insulin or Oral Hypoglycaemic drugs, showed an increased number of amino acids excreted in their urine.

While comparing the excretion of amino acids in NI and DP, A significant difference ($z -3.8062$) was found in their percentage, excreting the same amino acid with the Mean of DP being 7.367, which is greater than the Mean of NI which is 5.50. This indicated that the percentage of DP excreting a particular amino acid was greater than the percentage of NI. This was particularly evident for the following amino acids: Asp in whom percentage of DP excreting these amino acids were more than percentage of NI While with respect to amino acids like Gly, Ala, Arg, Thr and Ser; percentage of DP was lesser than that of NI.

Semi-quantitative estimation of five amino acids (Glu, His, Met, Phe, Ile), done by comparing the developed spot area (sq cm) of the respective amino acid in the chromatogram of Normal individuals and Diabetic patients with that of the standard chromatogram of 5 amino acids; revealed a significant difference in the approximate concentration of Glu ($z = -7.2128$), His ($z = -5.198$) and Met ($z = -2.464$). In these 3 amino acids, mean concentration in Diabetic patients was found to be higher than Normal individuals. For e.g. In case of Methionine (Mean of DP: 2.085 $>$ Mean of NI: 0.916), His (Mean of DP: 9.028 $>$ Mean of NI: 1.640) and Glu (Mean of DP: 53.471 $>$ Mean of NI:12.882). This indicated that the concentrations of these amino acids were higher in DP than that of NI. While, though in case of Phe, Mean of DP: 5.624 $>$ Mean of NI:0.906 and in case of Ile, Mean of DP: 2.46 $>$ Mean of NI:0.207; which meant that the concentrations of these amino acids are greater in DP than NI, yet according to their z score, Phe ($z = -2.1465$), and Ile ($z = -0.045$), this difference was not of much significance.

This observation was in accordance with the study conducted by R.M. Salek & M.L. Maguire *et al.*, who observed an increase in amino acids Tryptophan, Leucine, Isoleucine, Valine & Histidine in the urine of Diabetic patients as

compared to normal healthy individuals.

Thus it is possible that urinary amino acid pattern in the DP is due to renal tubular dysfunction in diabetic subjects and that urine amino acid patterns could serve as an inexpensive marker for the development of nephropathy; though further research needs to be conducted in large scale, with more sample size, separately for different types of Diabetes and also in people who are prone to become Diabetic; so that an early diagnosis can be achieved long before the appearance of clinically overt symptoms of Diabetes.

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