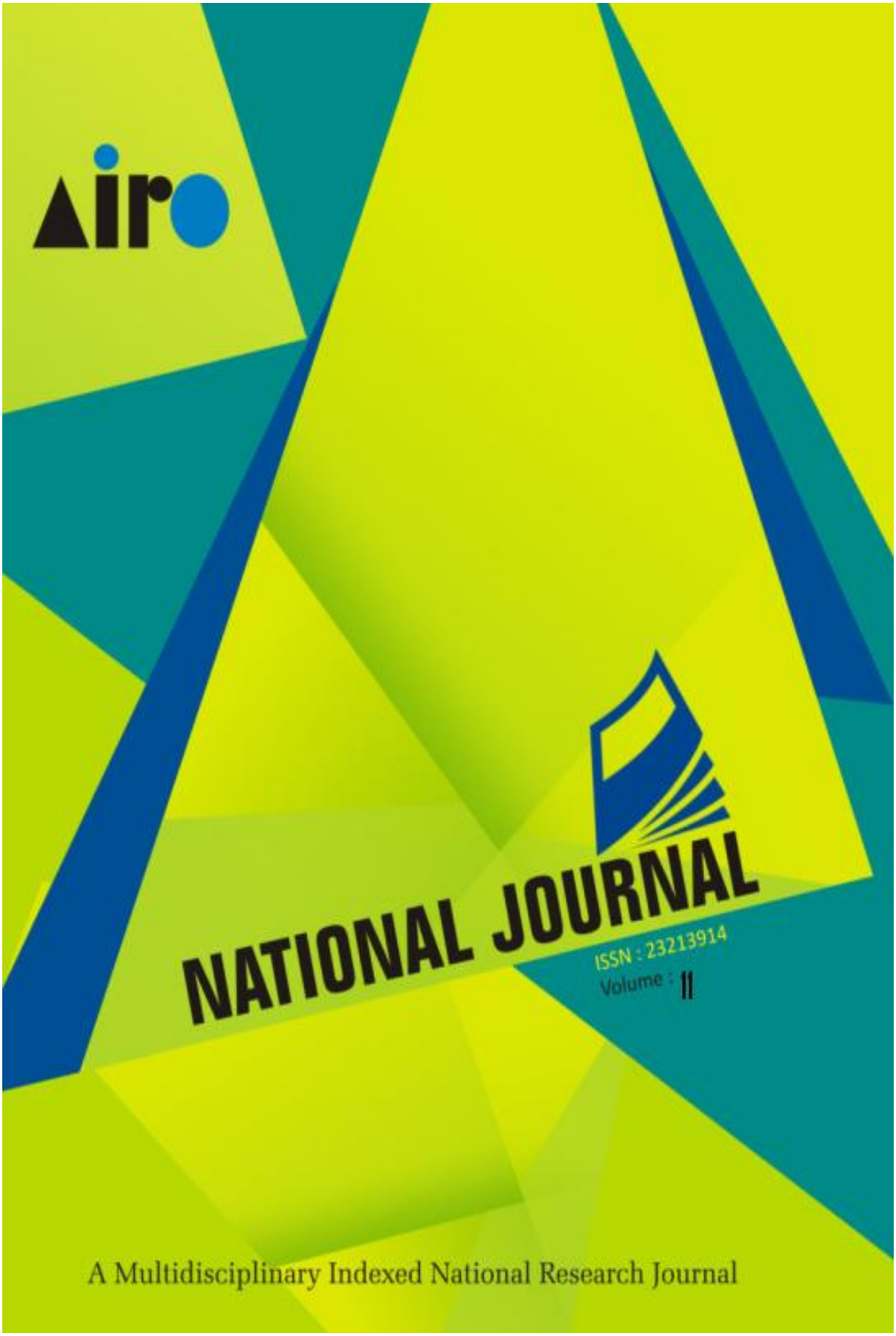
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Amino acids excreted in urine of Diabetic patients compared to normal individuals

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ABSTRACT

The high prevalence, chronicity, incurable nature and long term implications on health care and health care costs have made Diabetes Mellitus a major cause of concern for patients worldwide. Therefore it is important to diagnose this disease as early as possible and maintain good control, so that the psychosocial implications, morbidity and mortality of this disease, which seriously erode the quality of life, can be prevented or reduced to a reasonable extent. The present study supports the reported relation that exists between the glycemic control of Diabetes and urinary amino acid losses, even in those who are under treatment. The metabolic significance of losses of amino acids being incurred in Diabetes can be considered as an index of prognosis of the disease. Chromatographic method of detection of amino acids is easy to perform, economical and has a good patient compliance, as only urine samples are required. Hence, screening of urine for amino acids can easily give information about metabolic control of Diabetes.

Key Words: amino acids, Diabetes mellitus, Chromatography, Protein.

INTRODUCTION

Amino acids are the smallest unit of proteins[12 (a)], which can be linked together in varying sequences to form a vast variety of proteins. Twenty-two amino acids are naturally incorporated into polypeptides and are called proteinogenic or standard amino acids. Some proteins may however contain additional amino acids that arise

by modification of amino acid already present in a peptide; which include methylation, formylation, acetylation, prenylation & phosphorylation of aminoacyl residues. Of these, 20 are encoded by the universal genetic code. Nine standard amino acids are called "essential" for humans because they cannot be synthesized by the human body, and so must be taken in the diet.[22] Humans can synthesize 12 of the 20 common amino acids from the amphibolic intermediates of Glycolysis & TCA cycle.[12(b)] These are known as nutritionally non-essential amino acids. All proteins in our body are made of L- α-amino acids with the exception of D-serine and D-aspartate found in Brain tissue.[12 (a).] Each amino acid contain a common central α Carbon atom to which a Carboxylic acid group, an amino group, a Hydrogen atom are covalently bonded and in addition the α Carbon atom is bound to a specific chemical group (R), that uniquely defines each of the 20 common amino acids. Besides these there is also derived amino acids.[30]

Free amino acids are absorbed across the intestinal mucosa by Sodium dependent active transport.[12(c).] Free amino acids filtered by the glomeruli are reabsorbed from the proximal tubule of nephron with the help of specific amino acid transporters transporting groups of specific amino acids.

Appearance of amino acids in urine is called Aminoaciduria. Amino acids are excreted normally in urine in very small amounts, but generalized aminoaciduria is seen in all types of Diabetic patients.[2]

The celebrated Greek physician Aretaeus the Cappadocian some 1900 years ago described diabetes as a condition with “a melting down of the flesh and limbs into urine.” Remarkably, his observations are amazingly durable and accurate even by the standards of today with reference to Type 1 diabetes.[23,9,33].

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]

Though it is established by various studies that 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.

AIMS & OBJECTIVES

Detect the type of amino acids excreted in the urine of all types of Diabetic patients undergoing treatment and measure the degree of aminoaciduria with control of Diabetes; compared to that of age and sex matched normal individuals by the method of Paper 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 in order to remove any proteinaceous substance present. The filtrate obtained was used for detection and estimation of amino acids by chromatography.

For Blood Glucose estimation: 2ml of fasting venous Blood samples were collected aseptically in an EDTA vial from all the participants for Fasting Blood Glucose estimation.

For estimation of Serum creatinine: 6ml of venous blood was collected aseptically in a plain vial and centrifuged. Proteins were precipitated by adding 2ml of Sodium Tungstate and 2ml of 2/3N Sulphuric acid drop by drop with constant shaking and allowed to stand for 10 mins. The supernatant fluid was used for estimation of creatinine.

For estimation of HbA1c: Random Capillary blood samples were collected aseptically in a vial containing mixtures

of Potassium Oxalate and Sodium Fluoride in the ratio 3:1, from diagnosed Diabetic patients for estimation of HbA1c.

MATERIALS USED

For Chromatography:

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

For Blood Glucose estimation:

1. Standard Glucose solution of conc. 100mg/dl
2. Glucose Oxidase Peroxidase
3. 4-Amino Antipyrine
4. 4-Hydroxy Benzoic acid

For estimation of Serum Creatinine:

1. Standard Creatinine Solution:
2. Saturated Picric acid solution
3. Sodium Hydroxide

For estimation of HbA1c:

- Test Device: A plastic device containing a membrane filter, 1
- R1 / Reagent**:** Contains Glycinamide buffer containing Zinc ions, Dye bound Boronic acid, Formamide (6.2%) and Sodium Azide (0.05%)
- R2 / Washing Solution: Contains Morpholine (50mmol/L) buffered Sodium Chloride solution, Sodium Azide (0.05%)

Nycocard Reader II

For detecting Glycosuria:

1. Tri-Sodium Citrate
2. Copper Sulphate
3. Sodium Carbonate

METHODS

Detection and Semi-Quantitative estimation of amino acids by Paper Chromatography

Detection of Amino acids:

Reagent Preparation: n-Butanol, Acetic acid & water were mixed in the ratio

12:3:5 and 0.1% Ninhydrin solution in Acetone was prepared.

Standardization: All the 20 synthetic amino acid solutions were applied separately as 4µl spots on the chromatography paper. The paper was then dipped in a solution of n-Butanol-acetic acid-water (12:3:5) in a chromatographic chamber. After development, the paper was allowed to dry for 30 minutes in room temperature and a 0.1% Ninhydrin solution was sprayed evenly over it, taking particular care that the series of spots of same amino acids were similarly sprayed and that no part of the paper was grossly wetted. The paper was left at room temperature for 30 mins after spraying and then inserted for exactly 2 mins into a heating apparatus at 100°c. The Rf values each amino acid were calculated by the formula: Rf= Distance moved by the solute / distance moved by the solvent. This paper formed the standard paper for Rf values.

Detection: Then the processed urine samples were applied as 4µl spots on chromatography paper, dipped in the solution of Butanol, Acetic acid & water and allowed to develop as above. Amino acids in the urine samples were identified by comparing the Rf values with those of the Standard paper.

Rf. Values obtained

Amino acids	**Rf. Value**	**Amino acids**	**Rf. values**
Ala	0.38	Leu	0.73
Arg	0.20	Lys	0.14
Asp	0.24	Phe	0.68
Cys	0.4	Pro	0.43
Met	0.55	Ser	0.27
Glu	0.30	Thr	0.35
Gly	0.26	Tryp	0.66
His	0.11	Tyr	0.45
Ile	0.72	Val	0.61

Table no. 1

Estimation of Serum Glucose level

Method: Glucose Oxidase Peroxidase method

Principle: Glucose reacts with Oxygen in presence of Glucose Oxidase to form Gluconic acid and Hydrogen peroxide. The Hydrogen peroxide then reacts with 4-Aminoantipyrine and 4-OH Benzoic acid in presence of Peroxidase enzyme to form a red coloured Quinoneimine dye. The intensity of colour is directly proportional to the concentration of Glucose present.

Procedure:

- In the 1st test tube, 1ml of GOD POD working reagent was added to 10µL of Distilled water and marked as "Blank" and Optical Density was set to "zero" at 505nm.
- In the 2nd test tube, 1ml of Working Reagent was added to 10µL of Standard Glucose solution and marked as "Standard" and Optical Density (OD) was measured at 505nm.
- In the third test tube, 1ml of Working Reagent was added to 10µL of Serum and marked as "Test" and Optical Density was measured at 505nm.
- Calculation: Concentration of Glucose = OD of Test / OD of Standard *Concentration of Glucose in Standard

Estimation of Serum Creatinine Level

Method: Jaffe's Method

Principle: Creatinine reacts with Picric acid in alkaline medium provided by Sodium Hydroxide to form an Orange-Red coloured complex. The intensity of colour is directly proportional to the concentration of Creatinine.

Reagent Preparation:

- Standard Creatinine Solution was prepared by adding 1.83ml of Conc. HCl to 500 ml of distilled water. Then to 100 ml of the above solution 0.1gm of Creatinine was added to obtain 1mg% of Standard Creatinine solution.
- 0.04M Picric acid solution was prepared by adding 9.16gm of Picric acid to 1L of Distilled water.

- 5% solution of Sodium hydroxide was prepared by adding 5mg of Sodium hydroxide to 100ml of Distilled water.

Procedure:

- In the 1st test tube, 3ml of Distilled water was taken and to it 1ml of Sodium Hydroxide and 1ml of Picric acid solution is added and marked as "Blank" and its Optical Density was set to "zero" at 520nm.

- In the 2nd test tube 3ml of this Creatinine Standard solution was taken and 1ml of Sodium Hydroxide and 1ml of Picric acid solution was added and marked as "Standard" and then its Optical Density was measured at 520nm.

- In the 3rd test tube, 3ml of Serum (Protein free filtrate) was taken and to it 1ml of Sodium Hydroxide and 1ml of Picric acid solution is added and marked as "Test" and then its OD was measured at 520nm.

- Calculation: Concentration of creatinine = OD of Test / OD of Standard *Concentration of Creatinine in Standard

Detection of Glycosuria:

Method: Benedict's Semi- quantitative test

Principle: Glucose is a reducing agent and hence it reduces the cupric ions of Benedicts Qualitative reagent to cuprous ions thus changing the colour from blue to brick red.

Reagent preparation: Benedict's Qualitative Reagent was prepared by adding 100gm of Tri-Sodium Citrate and 100gm of Sodium Carbonate to 500ml of Distilled water. Then 175gm of Copper Sulphate was added to 100 ml of Distilled water. Both the solutions were mixed and volume was made up to 1L

.Procedure:.

1. 2ml of urine was added to 5ml of Benedicts Qualitative reagent and heated for 2 minutes.

2. Presence of Glucose was indicated by greenish to brick red discolouration of the Benedicts reagent. No change in colour indicated absence of glucose in urine.

Estimation of Glycosylated Haemoglobin (HbA1c)

Principle: Nycocard-HbA1c is a rapid in vitro test for measurement of Glycated Haemoglobin in human blood. It has a measuring range of 3-18% of HbA1c, measuring interval of 0.1% and reference range of 4.3-6.3% HbA1c.

Glycated Haemoglobin differs from non-glycated haemoglobin by the attachment of a sugar moiety at the N-terminal Valine residues of β-chain of Haemoglobin by means of a ketoamine bond. Glycated Haemoglobin thus contains 1,2-cis-diol groups not found in non-glycated haemoglobin. These diol groups provide the basis for separation of glycated from non-glycated components by Boronate Affinity Chromatography.

When blood is added to the reagent, the erythrocytes undergo lysis immediately and precipitation of all haemoglobin occurs. The Boronic acid conjugates then bind to cis-diol group of Glycated haemoglobin. An aliquot of the reaction mixture is added to the Test Device and all the precipitated Haemoglobin, bound and unbound, remains on the top of the filter. Any excess of coloured conjugate is removed with the washing solution. The precipitate is then evaluated by measuring the 'Blue' (Glycated Hb.) and the 'Red' (Total Hb.) colour intensity with the Nycocard Reader II; the ratio between them being proportional to the percentage of HbA1c in the sample.

RESULT

❖ OBSERVATIONS

DETECTION OF AMINO ACIDS

Amino acids detected in the urine of normal individuals (1-15) Table no. 2

Amino acids **Sample No.**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Gly	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Ala	nd	D	D	nd	D	nd	D	nd	nd	nd	nd	nd	D	nd	nd
Val	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Leu	nd	nd	nd	nd	nd	nd	nd	D	nd	nd	nd	nd	nd	nd	nd
Ile	D	nd	nd	nd	D	D	D	D	D	D	D	D	nd	D	nd
Ser	nd	D	nd	D	nd	nd	D	D	nd	nd	nd	nd	nd	nd	nd

Thr	nd	nd	nd	D	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cys	nd	D	nd	nd	D	D	D	D	nd	nd	nd	nd	nd	nd	nd
Met	D	nd	nd	nd	nd	nd	D	D	D	D	nd	D	D	D	D
Asp	D	D	D	nd	nd	nd	nd	nd	nd	nd	D	nd	nd	nd	nd
Arg	D	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lys	nd	nd	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
His	D	nd	D	nd	D	D	D	D	nd	D	nd	nd	nd	D	nd
Phe	nd	D	nd	D	nd	nd	D	nd	nd	nd	nd	nd	nd	nd	nd
Tyr	nd	nd	D	nd	nd	nd	nd	nd	nd	nd	D	nd	nd	D	nd
Tryp	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pro	nd	nd	nd	D	nd	nd	nd	D	D	D	nd	D	nd	nd	nd
Gln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Glu	nd	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Amino acids detected in the urine of normal individuals (16-30)

Amino acids **Sample No.**

	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Gly	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Ala	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Val	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	D	nd	nd	nd	nd
Leu	nd	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ile	D	nd	D	D	nd	nd	nd	D	nd	D	nd	D	D	nd	nd
Ser	nd	nd	D	nd	nd	D	nd	nd	nd	nd	nd	nd	nd	D	nd
Thr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Cys	D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Met	D	D	D	D	D	D	D	D	D	D	nd	D	D	nd	nd
Asp	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lys	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
His	nd	nd	nd	nd	D	nd	nd	D	nd	D	nd	nd	nd	nd	nd
Phe	nd	nd	nd	nd	nd	D	nd	nd	nd	nd	nd	D	nd	nd	nd
Tyr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	D	nd
Tryp	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	D
Pro	nd	D	nd	nd	nd	D	D	D	D	D	D	D	D	D	D
Gln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Glu	D	D	D	nd	D	D	nd	nd	D	D	D	D	D	D	D

Table No. 3

Amino acids detected in Diabetic Patients (1-15)

Amino acids **Sample No.**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Gly	nd	D	D	D	nd	D	nd	nd	D	D	D	D	D	D	D
Ala	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Val	nd	nd	nd	D	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	D
Leu	nd	nd	nd	D	nd	nd	nd	nd	nd	nd	Nd	D	nd	nd	D
Ile	D	D	D	D	D	nd	D	D	D	D	D	D	D	D	D
Ser	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Thr	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd

Cys	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Met	D	nd	nd	D	nd	D	D	nd	D	D	D	D	D	D	D
Asp	D	D	D	D	D	D	D	D	nd	D	D	D	D	nd	D
Gln	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Glu	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Arg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Lys	nd	nd	nd	D	nd	D	nd	nd	D	nd	Nd	D	nd	nd	D
His	nd	D	D	D	D	D	D	nd	D	D	D	D	D	D	nd
Phe	nd	nd	D	D	D	D	nd	nd	D	nd	D	D	D	nd	D
Tyr	nd	nd	nd	nd	nd	nd	nd	nd	D	nd	D	D	D	nd	D
Tryp	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
Pro	D	D	D	D	D	D	D	nd	D	D	D	D	D	D	D
Asn	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd

Table No.4

Amino acids detected in Diabetic Patients (16-30)

Amino acids **Sample No.**

	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Gly	D	D	D	nd	nd	D	D	nd	D	D	D	D	D	nd	D
Ala	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Val	nd	nd	nd	nd	nd	nd	nd	nd	D	nd	nd	nd	nd	nd	nd
Leu	nd	nd	nd	nd	nd	nd	nd	nd	Nd	D	nd	nd	nd	nd	D
Ile	D	D	D	D	D	D	D	D	D	D	D	D	D	nd	D
Ser	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Thr	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd

Cys	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Met	D	nd	D	D	D	D	nd	nd	Nd	D	D	D	D	D	D
Asp	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Gln	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Glu	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Arg	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Lys	nd	nd	D	D	D	D	D	D	D	D	nd	nd	nd	nd	D
His	nd	D	D	D	D	nd	D	D	D	D	D	D	D	D	D
Phe	nd	D	D	D	D	nd	D	D	D	D	nd	nd	D	nd	D
Tyr	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
Tryp	nd	D	D	D	D	nd	nd	nd	D	D	nd	nd	nd	nd	nd
Pro	D	nd	D	D	D	D	nd	nd	Nd	D	D	D	D	nd	D
Asp	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	Nd

Table No.5

SUPPORTING PARAMETERS OF

NORMAL INDIVIDUALS

Subjects	**Fasting Blood Glucose Level (mg/dl)**	**Serum Creatinine level (mg/dl)**	**Presence of Glucose in Urine**
1	70	0.8	absent
2	75	0.7	absent
3	80	0.9	absent
4	68	0.9	absent
5	90	1.12	absent
6	72	0.8	absent
7	70	0.8	absent
8	65	1.13	absent

9	73	1	absent
10	80	1	absent
11	75	1.1	absent
12	70	0.8	absent
13	71	0.7	absent
14	69	0.6	absent
15	78	0.8	absent
16	81	1.12	absent
17	67	0.8	absent
18	74	1.14	absent
19	72	1.04	absent
20	79	1	absent
21	77	1.06	absent
22	67	1.03	absent
23	71	1.15	absent
24	72	1	absent
25	68	0.9	absent
26	70	1	absent
27	70	1.03	absent
28	65	1.17	absent
29	82	1.14	absent
30	75	1.16	absent

Table No.6

SUPPORTING PARAMETERS OF

DIABETIC PATIENTS

Subjects	**Fasting Blood Glucose Level (mg/dl)**	**Serum Creatinine level(mg/dl)**	**Presence of Glucose in Urine**	**Glycosylated Hb (HbA1c) In %**
1	135	.9	present	6
2	166	1.16	present	6.5
3	170	1	present	7.9
4	250	1.25	present	12
5	180	1.05	present	7.5
6	200	1.17	present	10.2
7	150	.9	present	6.8
8	127	.8	absent	5.9
9	200	1.17	present	9.8
10	160	.8	present	6.8
11	220	1.18	present	11.2
12	250	1.18	present	12.1
13	180	0.8	present	9.4
14	132	0.6	absent	5.9

15	190	1.09	present	10
16	129	1	absent	5.1
17	130	0.9	present	6.8
18	240	1.12	present	11.9
19	200	1.11	present	10.6
20	192	1.12	present	10.1
21	130	0.8	absent	6
22	140	0.9	present	6.2
23	128	0.7	absent	6
24	170	1	present	8.2
25	235	1.18	present	11.4
26	140	0.7	present	6.8
27	180	1	present	8.2
28	160	0.8	present	6.3
29	128	0.6	absent	6
30	180	1	present	9.7

Table No.7

STATISTICAL ANALYSIS

Comparison of number of amino acids excreted in Diabetic patients with their Fasting Blood Glucose Levels (mg/dl)

No. of amino acids	Fasting Blood Glucose (mg/dl)	No. of amino acids	Fasting Blood Glucose (mg/dl)
2	127	8	140
4	128	8	160
4	129	9	170
5	132	9	180
5	135	9	190
6	128	9	192
6	130	9	200
6	150	9	200
6	166	9	220
6	180	10	180
7	130	10	200
7	140	10	250
7	160	11	235
7	170	11	240
7	180	11	250

Table No.8

Co-relation (R) between the No. of amino acids (aa) excreted and Fasting Blood Glucose level (FBG) was derived by the formula:

R = Covariance (aa,FBG) / Standard Deviation of aa*Standard Deviation of FBG

Value of R=0.79, which indicated positive correlation between number of aa excreted and their FBG values. Thus we can conclude that in this case, the degree if aminoaciduria in DP increased with the increase in their Fasting Blood Glucose level. The co-relation of degree of aminoaciduria in DP with their Fasting Blood Glucose level is represented graphically in Fig.21

Comparison of Number of amino acids detected in 30 Diabetic patients with their HbA1c values

Number of amino acids detected	HbA1c Values (%)	Number of amino acids detected	HbA1c Values (%)
4	5.1	7	7.9
2	5.9	6	8.2
5	5.9	8	8.2
5	6	8	9.4
5	6	10	9.7
6	6	9	9.8
7	6	9	10
8	6.2	9	10.1
7	6.3	9	10.2
6	6.5	10	10.6
6	6.8	9	11.2
7	6.8	10	11.4
7	6.8	11	11.9
7	6.8	10	12
6	7.5	11	12.1

Table No.9

Co-relation (R) between the No. of amino acids (aa) excreted and HbA1c level was derived by the formula:

R = Covariance (aa,HbA1c) / Standard Deviation of aa*Standard Deviation of HbA1c

Value of R=0.89, which indicated positive correlation between number of aa excreted and their HbA1c values. Thus, in this case, the degree of aminoaciduria in DP increased with the increase in their HbA1c level. Hence we can say that degree of aminoaciduria increased with poorer control of Diabetes.

DISCUSSION

This study describes the identification of different amino acids in the urine of Diabetic patients compared to that of normal individuals. Chromatograms of amino acids detected in urine samples collected from both male and female normal individuals in the age group 25 to 40 years in Table No.2 and 3. It was seen that normal individuals exhibited generalized aminoaciduria. Gly was found to be excreted in all normal individuals (100%), probably due to its lowest molecular weight and simplest structure. Other amino acids excreted in large percentage of individuals were Glu (86.67%), Met (70%), Ile (56.67%) and Pro (50%). Lys and Val were found to be excreted in least number of individuals (3.33% each), Arg, Leu Tryp and Thr were excreted in 6.67% each, Asp and Tyr (13.33% each), Phe (16.67%), Ala and Cys (20% each), Ser (23%) and His (36.67%). of normal individuals.

Chromatograms of amino acids detected in urine of diabetic patients of both Type 1 & 2 undergoing treatment in the form Insulin and other Oral Hypoglycaemic medication, within the age group 25 to 40 are shown listed in Table No.4 and 5.

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. Amino acids which were not detected in Diabetic patients are Ala, Arg, Cys, Ser and Thr. This finding was partly in accordance with the observation of *R. Krishnaprasad* [26], who found that, in a group of patients with type 2 diabetes the amino acid excretion pattern showed that a greater proportion of Isoleucine, Cysteine,

Arginine, Serine, Threonine, Proline, Leucine, Alanine, Glycine, Glutamic acid, Methionine, Tyrosine and Lysine in that order of frequency which contrasted sharply with the pattern in normal people where Alanine and Glycine are the main constituents. The difference in the observation of pattern of aminoaciduria may be attributed to the fact that the study conducted by *R.Krishnaprasad* included only those DP whose Diabetic status were under control (Fasting Blood Glucose level < 110mg/dl), while this present study included all the DP under treatment, irrespective of their Fasting Blood Glucose level.

It was also observed that number of amino acids excreted in urine of DP has a positive co-relation with their Fasting Blood Glucose level (R=0.79), which was partly in accordance with the finding of *R. Krishnaprasad* [26], which stated that; while a trend of increasing amino acid loss with high blood glucose was noted, there was poor correlation between the amount of amino acid lost in the urine and the blood glucose levels (R= 0.245).

A positive co-relation of aminoaciduria with Glycosuria was also found. Less number of amino acids was found to be excreted in patients (minimum of 2 and maximum of 5) in whom Glycosuria was absent. This observation was in accordance with the Study by *Bingham*[15] who concluded that there is increasing aminoaciduria with increasing degrees of Glycosuria, the probable reason being that glucose in the renal tubules is believed to depolarize and dissipate the electrical gradient of the sodium dependent glucose transporters.

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