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A pilot study on screening for aminoacidurias using paper chromatography in subjects with type 2 diabetes and healthy subjects: Cross-sectional study

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ABSTRACT

Introduction: Diabetes is a heterogeneous chronic dysglycemic and dyslipidemic metabolic disorder principally characterized by persistent hyperglycemia resulting from defects in insulin action and/or insulin secretion. This pilot study is indented detect type of Aminoacidurias and number of amino acids detected in the urine and correlate number of amino acids detected with glycemic control marker glycated hemoglobin (HbA1c) in type 2 diabetic subjects and healthy controls.

Materials and Methods: This present study included 40 subjects with type 2 diabetes and 40 age and gender matched healthy controls in the age group 25-60 years. Early morning first voided mid-stream urine sample of 5 mL was collected into the urine container and subjected to centrifugation to remove cell debris and the clear supernatant was used for preforming paper chromatography for detecting aminoacidurias.

Results: In type 2 diabetic subjects two or more than two amino acids up to seven are detected in the urine sample in comparison to healthy controls. The most commonly detected amino acids were valine (60%), glycine (45%), leucine (32.5%), alanine (22.5%) and histidine (25%). There was statistically significant strong correlation existed between HbA1c levels and number of amino acids detected in type 2 diabetic subjects (r=0.901). We found statistically significantly elevated levels of HbA1c and fasting plasma glucose levels in type 2 diabetic subjects compared to healthy controls.

Conclusion: In type 2 diabetic subjects two or more than two amino acids were excreted in comparison to controls. It was observed that those patients with inadequate glycemic control had more number of amino acids detected in the urine evidenced by strongly correlation with HbA1c values. Detection of amino acids in the urine can be used as a non-invasive tool to predict glycemic control in type 2 diabetic patients. In addition to this, in type 2 diabetic subjects we get an information about the deficient amino acids and increased amino acids which can open the gateway for therapeutic modalities using amino acids.

1. Introduction

The major non-communicable chronic dysglycemic metabolic disease affecting an individual from head to toe is none other then diabetes mellitus. It is quite alarming from inside out affecting our body physiologically, metabolically, emotionally and mentally from head to toe not sparing even a single cell in our body. Diabetes is a heterogeneous chronic dysglycemic and dyslipidemic metabolic disorder principally characterized by persistent hyperglycemia resulting from defects in insulin action and/or insulin secretion.[1]

According to data from ICMR-India Diabetes 2023, 130 million Indians have diabetes at present, and an additional 136 million are in the pre-diabetes stage. Diabetes primarily comes in two forms: type 1 diabetes, which is primarily found in younger age groups and is caused by autoimmunity attacking the pancreas, and type 2 diabetes, which is more

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common in obese people and is primarily caused by insulin resistance as the underlying mechanism for dysglycemia.[2]

Initially, diabetic patients' urine samples were tasted to diagnose the disease; urine glucose detection and blood glucose measurement followed. The detection of glucose levels in blood, serum, and plasma has advanced significantly from the colorimetric approach to point-of-care testing with glucometers (dry chemistry). Since blood glucose levels fluctuate from day to day and from time to time and are also influenced by recent meals, glycated hemoglobin, or HbA1c, has emerged as a fantastic diagnostic and prognostic marker that represents average glycemic control over the previous three months. We require an EDTA whole blood sample, which is an intrusive and expensive test, to perform the HbA1c test. Hence we need an non-invasive, economical biomarker test which can decide the patients glycemic control, it will be of a great help to the patients suffering from type 2 diabetes.

Amino acids are building blocks of proteins having both amino group and carboxyl group. There are about 22 amino acids total, of which the human body cannot synthesis nine, making them extremely necessary. A variety of hormones, including insulin, nucleotides, lipids, signaling molecules, and metabolic intermediates involved in the production of energy are synthesized using amino acids. Two important functions of amino acids in the production of energy are 1) Through a process known as anaplerosis, they are changed into an intermediate of the Krebs cycle, which keeps the cycle active. 2) A range of organ systems can produce fuels through the mobilization of amino acids.[3–14]

The intestinal mucosa uses sodium-dependent active transport to absorb free amino acids. Specific amino acid transporters assist in the glomeruli's filtration of free amino acids, which are then resorbed from the proximal tubules of the nephron. A healthy person's plasma amino acid concentration is about 1 mmol/L when they consume 1-2 g of protein per kg of body weight from their diet. Excessive plasma concentration filtration, widespread tubular damage, or heritable abnormalities in the amino acid transport pathway all contribute to increased renal excretion of amino acids.

Normal urine typically contains the highest concentration of glycine, followed by histidine, glutamine, and serine. The content of branched chain amino acids may be a precursor to diabetes. Aminoaciduria is the term for the excretion of measurable levels of amino acids in urine. Urine normally excretes relatively modest amounts of amino acids. Every kind of diabetic patient has generalized aminoaciduria.[15] When the name "diabetes" was first used in 400–500 BC, Cappedocia said that it was an uncommon condition that was marked by the wet and cold melting of limbs and flesh into urine.[16–22]

Because protein breakdown in diabetic patients with insulin deprivation is more extensive than protein synthesis, there is a net loss of protein in the urine during insulin deprivation, which leads to a net release of amino acids.[23–27] The present study is intended to detect amino acids in urine of type 2 diabetic patients and to correlate number of amino acids excreted in urine with HbA1c levels.

2. Aim

To study Aminoacidurias in type 2 diabetic patients and healthy controls.

3. Objectives

1. To detect and compare the type of amino acids excreted in urine of type 2 diabetic patients and healthy controls using paper chromatography.
2. To correlate number of amino acids excreted with HbA1c levels in type 2 diabetic patients irrespective of their therapeutic modalities and duration of diabetes.

4. Materials and Methods

4.1. Study design and study settings

Cross-sectional study on "Screening for Aminoaciduria using Paper Chromatography in Subjects with Type 2 Diabetes and Healthy Subjects" was conducted in the department of Biochemistry in collaboration with Department of General Medicine, National Institute of Medical College & Research. This pilot study included a total of 40 type 2 diabetic subjects who are on treatment and 40 healthy controls in the age group 25-60 year of both the genders. We excluded the patients with type 1 diabetes, urinary tract infections, renal failure, diabetic nephropathy, renal glycosuria and known cases of inborn errors of metabolism.

4.2. Sample collection and biochemical analysis

Whole blood and plasma: under aseptic precautions 2 mL of venous blood sample was collected in EDTA vial after overnight fasting and used for estimation of glycated hemoglobin (HbA1c) by HPLC method and the sample is subjected for centrifugation for the separation of plasma used for fasting blood glucose estimation by Glucose Oxidase-Peroxidase method (GOD-POD method) in fully automated integrated biochemistry analyzer (Vitros 5600). Urine: Early morning first voided mid-stream urine sample of 5 mL was collected into the urine container and subjected to centrifugation to remove cell debris and the clear supernatant was used for paper chromatography.

Detection of Amino Acids by Paper Chromatography: Reagents required: 1) Solvent was prepared in the ratio of 12:3:5 using Butanol: Acetic Acid: Water. 2) Staining amino acids: 0.1% Ninhydrin solution in Acetone. Procedure: Standardization: All 20 synthetic amino acid solutions were applied separately as 3 micro liter spots on chromatography

paper allowed to dry, and the paper was dipped in the chromatography chamber containing the solvent (12:3:5). 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 Retention Factor (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 of type 2 diabetic subjects and controls 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.

4.3. Ethical consideration and consent

The study was conducted after taking Institutional Ethical Committee clearance with the number Reference No EC/New/Inst/2022/RJ/0118 and Proposal Number IEC/P-427/2023. Informal written consent was obtained from the all the subjects (type 2 diabetic subjects & healthy controls) involved in the study. All methods were performed in accordance with the relevant guidelines and regulations.

5. Results

In the present study, 40 patients with type 2 diabetes and 40 healthy controls were included as per inclusion and exclusion criteria. The mean age in type 2 diabetic subjects was 48.7±10.75 years and in healthy controls 47.54±11.22. Out of 40 type 2 diabetic subjects 22 were males and 18 were females. Similarly in 40 healthy subjects 26 were males and 14 were females. The mean levels of glycated hemoglobin (HbA1c) and fasting plasma glucose were statistically significantly elevated in type 2 diabetic subjects compared to healthy controls ($p<0.001$) and there was no significant differences in creatinine levels between type 2 diabetic subjects and healthy controls as depicted in Table 1.

Table 2 shows the urinary paper chromatography for amino acids findings in healthy controls. It is seen that among 40 healthy controls, single amino acid was detected in 22 controls. The pattern of amino acids detected were Glutamic acid in 10 controls, Arginine in 4 controls, Cysteine in 2 controls, Valine in 3 controls, Glycine in 3 controls.

Table 3 shows in type 2 diabetic subjects more than two amino acids were detected in the urine (minimum of two and maximum of 7). Alanine was detected in 9, Arginine in 7, Glycine in 18, Glutamic acid in 24, Histidine in 10, Isoleucine in 1, Lysine in 6, Leucine in 13, Valine in 24, Phenyl alanine in 4, Tyrosine in 5, Tryptophan in 2, Proline in 5, Serine in 4, Methionine in 3. The most commonly detected amino acids were valine (60%), glutamic acid (60%), glycine (45%), leucine (32.5%), alanine (22.5%) and histidine (25%).

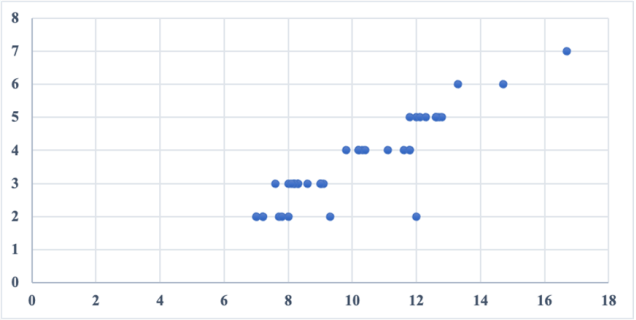

Figure 1: (X-axis: HbA1c levels,Y-Axis: Number of Amino Acids Detected)
Scatter plot shows the correlationbetween HbA1c and Number of Amino Acids Excreted in type 2 diabetic subjects

Table 4 and Figure 1 The pearsons correlation coefficient was applied between HbA1c and number of amino acids detected, in type 2 diabetic subjects. It is found that there existed a strongly positive correlation with a r value of 0.901 and it was statistically highly significant.

6. Discussion

The present study intended to screen for urinary amino acids using paper chromatography in type 2 diabetic subjects and healthy controls. Based on inclusion and exclusion criteria, 40 type 2 diabetic subjects and 40 healthy controls were enrolled. Out of the 40 type 2 diabetic subjects 22 were males and 18 were females, similarly 24 were males and 16 were females in healthy controls. The baseline parameters fasting blood glucose (FBG), glycated haemoglobin (HbA1c) and serum creatinine levels were compared. It was found that the fasting blood glucose and glycated haemoglobin levels were statistically significantly elevated in type 2 diabetic subjects compared to healthy controls ($p<0.001$) and there was no significant differences in the levels of serum creatinine between type 2 diabetic subjects and healthy controls as depicted in Table 1.

Aminoacidurias are detected using paper chromatography technique, out of the forty healthy controls amino acids were detected in twenty two controls. The pattern of amino acids detected were Glutamic acid in 10 controls, Arginine in 4 controls, Cysteine in 2 controls, Valine in 3 controls, Glycine in 3 controls in Table 2. In type 2 diabetic subjects more than two amino acids were detected in the urine (minimum of two and maximum of 7). Alanine was detected in 9, Arginine in 7, Glycine in 18, Glutamic acid in 24, Histidine in 10, Isoleucine in 1,

Table 1: Shows demographic profile of the type 2diabetic subjects and healthy controls included in the study

	Type 2 Diabetic Subjects (no =40)	Healthy Controls (n=40)	p value
Age in years	48.7±10.75	47.54±11.22	
Males/Females	22/18	26/14	
HbA1c values	9.277±2.848	5.23±1.22	<0.001
Fasting blood glucose (mg/dL)	273.65±208.3	82.64±9.42	<0.001
Creatinine (mg/dL)	0.639±0.48	0.632±0.49	

Lysine in 6, Leucine in 13, Valine in 24, Phenyl alanine in 4, Tyrosine in 5, Tryptophan in 2, Proline in 5, Serine in 4, Methionine in 3. It is evident that the most common amino acids detected were Valine and Glutamic acid and Glycine as represented in Table 3. The pearsons correlation coefficient was applied between HbA1c and number of amino acids detected, in type 2 diabetic subjects. It is found that there existed a strongly positive correlation with a r value of 0.901 as depicted in Table 4 and Figure 1 and it was statistically highly significant.

Interrelation between amino acids and type 2 diabetes mellitus: In the realm of regulating glucose metabolism, one of the most active areas of research is the functions and underlying mechanisms of different amino acids in connection to type 2 diabetes. A major factor in prediabetes and the chance of developing diabetes in the future is the dysregulation of amino acid metabolism. The relationships between branched-chain amino acids, aromatic amino acids, tryptophan, glycine, asparagine, and aspartate, as well as T2DM, are further explained in this section.

Branched chain amino acids in type 2 diabetes mellitus: they include valine, leucine and isoleucine, and are nutritionally essential amino acids derived from the protein containing foods[28,29] BCAAs significantly modify and regulate a variety of physiological and metabolic processes, such as glucose, lipid, or energy balance, through a specific signalling network.[30] Raised plasma BCAA levels and type 2 diabetes have been found to consistently correlate in both human[31,32] and rodent models.[33,34] Elevated plasma BCAA levels in diabetes individuals with poor insulin signalling were originally reported in the early 1970s.[35] Elevated blood levels of BCAAs have also been linked to increased insulin resistance, homeostasis

model assessment (HOMA), insulin sensitivity, and HbA1c level, according to recent large prospective and cross-sectional cohort studies.[36] It was discovered that higher plasma BCAA levels were positively connected with fasting insulin levels but negatively correlated with insulin sensitivity.[37] Because of these consistent outcomes, there has been conjecture over BCAAs' possible causal significance.[38] In the present study we observed in 24 (60%) patients valine was detected, in 13 (32.5%) patients leucine and in one patient (2.5%) isoleucine was detected. The most common being valine. Due to higher concentration these amino acids in the serum and crossing the renal threshold they are excreted in the urine are detected using paper chromatography. Numerous mechanisms suggest a clear correlation between BCAAs and the gluconeogenic properties of valine and isoleucine, while leucine and isoleucine exhibit insulinotropic actions. Through the activation of the mammalian target of rapamycin complex 1 (mTORC1), leucine can impact the activity of the insulin receptor, while insulin is responsible for mediating the branched-chain-ketoacid dehydrogenase complex (BCKDH). Researchers continue to debate whether BCAAs are actual causes of insulin resistance and type 2 diabetes or just passive indicators of reduced insulin action, despite mounting evidence confirming the predictive power of elevated BCAA levels in T2DM.[39] Glycine in type 2 diabetes mellitus: Glycine is an non-essential glycogenic amino acid synthesised in the body involved in various metabolic pathways and synthesis of biologically important compounds especially Glutathione which respires the strongly antioxidant system in our body. Recently one study found that, decreased plasma glycine content is thought to be a promising predictor of decreased glucose tolerance and type 2 diabetes. Higher serum glycine levels are associated with a lower risk of incidence type 2 diabetes, according to prospective studies, and baseline hypoglycemia may signal a predisposition to the disease. When compared to control subjects, patients with diabetes or obesity showed a relatively lower plasma glycine concentration.[40] In particular, this metabolic alteration takes place prior to the disorder's evident clinical signs. Furthermore, according to the homeostasis model assessment for the beta cell function index, there is a positive correlation between the amount of plasma glycine with insulin sensitivity and a negative correlation with insulin resistance.[41] Additionally, compared to placebo, diabetic patients receiving insulin-sensitizer therapy, which includes pioglitazone and metformin, had greater plasma glycine levels.[42] A 4C investigation, however, did not find any noteworthy alterations in the ORs of glycine prior to the onset of glucose dysregulation in the prospective population.[23] This suggests that more research be done to see whether glycine may be used as a clinical diagnostic tool for type 2 diabetes. In the present study, Glycine was detected in 18 (45%) patients who had HbA1c range of 6.5 to 7.6. Glutamic acid in type 2 diabetes mellitus: Glutamic acid is an acidic non-essential glucogenic amino

Table 2: Shows Urine Amino Acids detected by paper chromatography in HealthyControls (D: Detected, ND: Not Detected)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Ala	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arg	ND	ND	ND	D	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND
Cys	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gly	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glu	D	D	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	D	ND	ND
His	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ile	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
Leu	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
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
Tyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trp	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	ND	ND	ND	ND	ND
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ala	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	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
Cys	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gly	ND	ND	ND	ND	D	D	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glu	ND	D	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	D
His	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ile	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
Leu	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
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
Tyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Val	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	31	32	33	34	35	36	37	38	39	40					
Ala	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Asp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Arg	ND	ND	D	ND	ND	ND	ND	ND	ND	D					
Cys	ND	ND	ND	ND	ND	D	ND	ND	ND	ND					
Gly	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Glu	ND	ND	ND	D	ND	ND	ND	D	D	ND					
His	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Ile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Lys	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Leu	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Ser	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Thr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Tyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Trp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Val	D	ND	ND	ND	D	ND	ND	ND	ND	ND					

Table 3: Shows urine amino acids detected by paper chromatography in type 2 diabetic subjects (D: Detected, ND: Not Detected)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Ala	ND	D	ND	D	ND	ND	ND	D	D	D	D	D	ND	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	D	ND	ND	ND	ND	ND	ND	ND	ND
Cys	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	ND
Gly	ND	ND	ND	ND	D	D	D	D	D	ND	ND	ND	ND	ND	ND
Glu	D	D	D	D	D	ND	ND	D	D	D	D	ND	D	ND	D
His	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	ND	ND
Ile	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lys	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	D	D	ND	D
Leu	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phe	ND	ND	ND	D	D	D	ND	ND	ND	ND	ND	ND	ND	D	ND
Ser	ND	ND	ND	D	ND	ND	ND	D	D	ND	ND	ND	D	ND	ND
Thr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tyr	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trp	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND
Val	D	ND	D	ND	ND	ND	ND	D	D	D	D	ND	D	D	D
Number of amino acids detected	2	2	4	4	3	3	3	5	5	3	4	3	4	2	3
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ala	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asp	ND	ND	D	D	ND	ND	ND	ND	ND	ND	D	D	ND	ND	ND
Arg	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	ND
Cys	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	ND	ND
Gly	ND	ND	D	D	D	D	ND	ND	D	D	D	ND	ND	ND	ND
Glu	D	D	ND	ND	ND	D	ND	D	D	ND	D	D	D	ND	D
His	ND	ND	D	D	ND	D	ND	D	D	ND	ND	D	ND	ND	ND
Ile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lys	D	D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Leu	ND	D	ND	ND	ND	ND	ND	D	D	D	D	ND	ND	ND	ND
Met	ND	ND	ND	ND	ND	D	ND	D	ND	D	ND	ND	ND	ND	ND
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	D	D	D	D	D
Phe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
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
Tyr	ND	ND	D	D	ND	D	ND	ND	D	ND	ND	ND	ND	ND	ND
Trp	ND	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	ND	ND	ND
Val	ND	ND	D	D	D	D	D	D	D	ND	ND	ND	D	D	D
Number of Amino acids detected	2	3	5	5	2	5	2	6	6	3	5	4	2	2	3
	31	32	33	34	35	36	37	38	39	40					
Ala	ND	ND	ND	ND	ND	ND	D	D	ND	ND					
Asp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Arg	D	D	D	ND	ND	ND	D	D	ND	ND					
Cys	D	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Gly	ND	ND	ND	ND	D	D	D	D	D	D					
Glu	D	D	D	D	D	ND	ND	ND	ND	ND					
His	ND	ND	ND	ND	ND	ND	ND	D	D	D					
Ile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Lys	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Leu	D	D	D	ND	D	D	D	D	ND	ND					
Met	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Phe	D	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Ser	ND	ND	ND	D	D	D	D	D	ND	ND					
Thr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Tyr	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Trp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND					
Val	D	D	D	ND	ND	ND	ND	D	D	D					
Number of amino acids detected	5	4	4	2	4	4	5	7	3	3					

Table 4: Shows HbA1c values and number of Amino acids excreted in type 2 diabetic subjects

HbA1c	Amino acids	HbA1c	Amino acids	HbA1c	Amino acids	HbA1c	Amino acids
7	2	10.2	4	12.6	5	12.8	5
7.2	2	8.6	3	8	2	11.8	4
9.8	4	11.6	4	13.3	6	11.8	4
11.1	4	9.3	2	14.7	6	7	2
8.2	3	8.3	3	9	3	10.2	4
8.3	3	12	2	12.7	5	10.4	4
9.1	3	8.2	3	10.3	4	12.6	5
12	5	12.1	5	7.7	2	16.7	7
11.8	5	12.3	5	7.8	2	8	3
7.6	3	7.2	2	9	3	8.1	3

acid synthesised in our body. It plays an important role in metabolic pathways and synthesis of biologically important compounds. Glutamic and aspartic acids have been linked to insulin resistance and secretion in earlier research. The production of glutamic acid[43] by adipocytes through the metabolism of branched chain amino acids (BCAAs) may account for the positive correlation shown between glutamic acid and visceral adipose tissue.[44,45] and waist circumference.[46] According to observations from other research, elevated plasma glutamic acid levels were linked to insulin resistance and aberrant fasting or 2-hour glucose levels. These findings imply that glutamic acid was connected with enhanced insulin resistance. Furthermore, nine amino acids, including glutamic acid and aspartic acid, were linked to higher glucose levels and lower insulin secretion, according to a recent population-based large cohort study.[47] In the present study, glutamic acid was detected in 24 (60%) patients. Aromatic amino acids in type 2 diabetes: Aromatic amino acids include phenyl alanine, tryptophan and tyrosine. Phenyl alanine and tryptophan are essential amino acids and tyrosine is non-essential amino acids. Aromatic amino acids are involved in the synthesis of important neurotransmitters, hormones, biogenic amines, catecholamines, pigment (melanin) and circadian rhythm (serotonin). It has been noted that phenylalanine and tyrosine, are associated with a propensity toward an elevated risk of type 2 diabetes.[48] Insulin resistance and signs of type 2 diabetes may develop in mice fed diets high in phenylalanine. Moreover, variations in an individual's fasting blood glucose (FPG) and two hours postprandial blood glucose (2hPG) levels closely correspond to variations in their phenylalanine and tyrosine levels. Additionally, it was shown that persons with normal glucose tolerance who acquired T2DM also had higher levels of these two AAAs. The study found that people who were not diabetic but had elevated levels of phenylalanine and tyrosine during hyperglycemia were more likely to acquire type 2 diabetes during a 5-year follow-up period.[48] In the present study, in 4 (10%) patients phenyl alanine was detected, in 5 (12.5%) patients tyrosine was detected and in 2 (5%) patients tryptophan was detected. Histidine, lysine and arginine in type 2 diabetes mellitus: In the present study, in 10 (25%) patients histidine as detected in 6 (15%) patients lysine was detected and in 7 (17.5%) patients arginine was detected. Proline, Serine, Methionine and Alanine in type 2 diabetes mellitus: In the present study, proline was detected in 5 (12.5%) patients, serine in 4 (10%) patients, methionine in 3 (7.5%) patients and alanine in 9 (22.5%) patients. It is thought that irregular serine metabolism plays a role in the etiology of type 2 diabetes and its associated problems, while opinions on the possible pathways are divided. It has been demonstrated that serine can improve glucose tolerance, raise insulin sensitivity, and stimulate insulin secretion.[48]

7. Conclusion

Particular amino acids are important early players in the development of incident insulin resistance and type 2 diabetes. Better amino acid utilization improves clinical outcomes and early diagnosis, delays the start and progression of type 2 diabetes, and permits preventative actions to be taken to prevent complications. Promising predictive metabolites Growing understanding of amino acid profiling has also spurred interest in a number of technologies since it may be used therapeutically to manage diabetes. Amino acids have amino acids hold both predictive and therapeutic potential in future type 2 diabetes. A thorough understanding of amino acid dysmetabolism in type 2 diabetes is essential for the effective screening, diagnosis and prediction of future diabetic complications, allowing clinicians to make informed decisions and benefitting individuals at risk. Once this diagnosis approach passes to the clinical level, it is expected to achieve considerably high detection accuracy and offer more specific therapeutic possibilities for high-risk patients. We found significant positive correlation between glycated haemoglobin levels and number of amino acids detected in type 2 diabetic patients. Detection of amino acids in urine can be used as a non-invasive tool to predict the glycemic control. Especially this will be a valid tool in type 2 diabetic patients with comorbid conditions like

anaemia and hemoglobinopathies, hypertriglyceredemia, acute blood loss, chronic liver failure, iron deficiency, uremias, alcoholism and hyperbilirubinemia where HbA1c values are not reliable.

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None.

9. Conflict of Interest

None.

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