Trend analysis of the correlation of amino acid plasma profile with glycemic status in Saudi diabetic patients

Fahad A. Al-Abbasi *

Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O. Box 50077, Jeddah 21523, Saudi Arabia

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Abstract The role of amino acids in diabetes mellitus and its metabolic traits have been suggested previously; however, studied to a very limited scale in the Saudi patient population. Patients diagnosed with diabetes mellitus were included in the current clinical study. Sample was representative and in accordance with the national population distribution. Blood samples were drawn and assayed for glucose, total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein. General biochemical markers, such as alkaline phosphatase (ALP), creatinine kinase (CK), aspartate transaminase (AST), alanine transaminase (ALT) and blood urea nitrogen (BUN) were assessed. Serum amino acids of different categories (essential, semi-essential and metabolic indicator amino acids) were assessed. Correlation co-efficient between each amino acid and serum glucose level was calculated. The current study showed positive correlation between amino acid level and glucose serum concentration in male while it showed negative correlation in female Saudi diabetic patients. Male patients had significantly higher methionine concentration parallel to their glycemic status. Metabolic indicator amino acids significantly changed in concordance with the glycemic status of female patients more than in male patients. In conclusion, serum amino acid is positively correlated with glycemic status in Saudi male diabetic patients while negatively correlated in female patients. Yet, further study would be recommended to utilize serum amino acid profile as surrogate parameter for the metabolic complications of diabetes mellitus.

Introduction Diabetes mellitus (DM) is a very common metabolic disease with expected worldwide burden of 300 million population in the year 2020. Kingdom of Saudi Arabia is classified level-4 DM disease burden with 717,000 patients in the year 2009 [1]. There have been changes over the last 20 years in the geographical distribution of the incidence of diabetes with significant decline in the industrialized countries and rise in the developing countries [2]. Kingdom of Saudi Arabia, a Middle
Eastern country, experienced significant economical improvement over the last 20 years. This has resulted in adopting the “Western” life style with respect to nutritional habits and physical exercise. Such changes might be coupled with improvement in health services; while on the other hand it could be associated with decline in the healthy eating habits and increase in the consumption of junk food [3].

DM is an insulin/glucose metabolic-endocrinal disease primarily characterized by elevated blood glucose and inability of glucose utilization in the peripheral tissues such as adipose and muscle tissues [4]. DM with both its major subtypes, insulin dependent diabetes mellitus and insulin independent diabetes mellitus, is accompanied by several vascular, neurological, immunological, and biochemical pathological changes [5]. Estimating early signs of these target organs pathological effect using non-invasive techniques represents an important issue for clinicians in order to avoid late organ depletions. Serum glucose and c-peptide encountered the major classic parameters to assess the glycemic and insulin efficiency statuses clinically [6]. In addition, several other biomarkers have been suggested for detailed or specific assessment of the diabetic status such as glucosenated hemoglobin for long term glycemic assessment [7].

Amino acids comprise the building blocks of proteins found in structural tissues of the body. Amino acids are essential to life in free or polymeric form as peptides. Amino acids play important roles in activities, such as neurotransmission, pH regulation, cholesterol metabolism, pain control, detoxification, and control of inflammatory response [8]. Many amino acids can be synthesized by the body. Others, the essential amino acids, cannot be endogenously synthesized in adequate amounts, so that they must be obtained from the diet [9]. The eight essential amino acids in humans are: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [10]. Semi-essential amino acids are those for which there is some endogenous synthesis, however, not enough for physiological needs. These include arginine, histidine, cysteine, and taurine (for infants) [11]. Other amino acids are considered metabolic indicators for several impairments in metabolism such as, beta-alanine, citrulline, homocysteine, cystathionine, ornithine, and alpha-aminoacidic acid. These amino acids provide information on conversion capability in the body or dietary adequacy of the essential amino acids [12]. Several references donated an important role of amino acids are a variety of many disorders, nonetheless DM. Some metabolic steps of amino acids related to vascular complications (methionine and arginine) exhibit a defective response to insulin in type-2 DM with nephropathy. However, gross alterations in the response of whole-body protein turnover are not evident in type-2 DM, specific investigations reveal subtle abnormalities in metabolic steps of selected amino acids [13]. Also it was reported that, circulating amino acid concentrations do not worsen the already attenuated glucose disposal in hyperglycemic type 2 diabetic men [14]. Also, specific amino acid or amino acid balance could be used as a predictive plasma marker for Type-2 DM [15].

Herein, in the current study on diabetic Saudi patients, we are assessing the correlation between the glycemic status of Saudi DM patients and their serum amino acid profile. This study might provide new insights into further metabolic interference of DM in Saudi population.

Patients and methods

Subjects

A total of 63 adult DM patients (27 males and 38 females) entered the study at a time after reading and accepting the institutional consensus. All participants were clinically diagnosed type-I DM according to the standard procedures within Saudi health care institutes (inclusion criteria). Any patient with combined sever illness or sever diabetic complications was excluded from the study (exclusion criteria). The study design was approved by the clinical ethics committee in Ministry of Health.

Clinical examination

Body mass index (BMI) was calculated as body weight (in kg) divided by squared height (in meters). The procedure for the measurements of weight, height, waist circumference and hip circumference, systolic and diastolic blood pressures was according to the standard procedures within Saudi health care institutes.

Collection of blood samples

Venous blood samples (two samples) were withdrawn from peripheral vein while the patient is sitting. Blood samples were allowed to clot, centrifuge and the sera were kept frozen at −20 °C for biochemical assessment. Another blood sample was collected in heparinized tube for amino acid analysis.

Serum glucose determination

Serum samples were used for glucose analysis on a glucose analyzer (Beckman Paragon, Fullerton, CA, USA). The method depends on the rate of oxygen consumption by the enzyme glucose oxidase in order to convert glucose into gluconic acid and hydrogen peroxide. Oxygen rate consumption was determined by sensitive oxygen electrode. The instrument was calibrated prior to glucose determination using quality control samples provided with the solutions. The mean values obtained on the controls were within the values quoted by the manufacturer. The results were expressed by SI units (mmol/l). The intra and inter assay coefficients of variation were 1.7% and 2.6%, respectively.

Biochemical assessments

Biochemical assessments were assessed in isolated sera using specific kits purchased from Dade Behring, Marburg, Germany.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed as previously described [18]. Creatinine was determined via picric acid chromophore interaction assay [19].

Creatinine kinase (CK), alkaline phosphatase (ALP) and blood urea nitrogen (BUN) were determined in sera using the manufacturer standard operating protocol of the kit.

Serum lipid analysis

Analysis was done using the Cobas Mira S Clinical Analyzer, Roche Diagnostics. Unimate 7 cholesterol and Unimate 7 triglyceride were used for measuring total cholesterol and triglycerides, respectively.
The assay for total cholesterol was done using the enzymatic method, which is an enzymatic colorimetric test with cholesterol esterase and cholesterol oxidase. Triglycerides were also assayed using an enzymatic colorimetric test with glycerol phosphate oxidase. A high density lipoprotein (HDL) cholesterol precipitating reagent, magnesium sulfate at a concentration of 0.26 mol/l was used to remove low density lipoprotein (LDL) cholesterol, total cholesterol and very low density lipoprotein (VLDL). HDL cholesterol was then determined by an enzymatic colorimetric method. LDL cholesterol was estimated by using the formula: LDL cholesterol = total cholesterol / (HDL cholesterol + 0.46 × triglyceride). The intra and inter-assay coefficients of variation were 2.2% and 2.2% respectively, for total cholesterol and 2.2% and 2.2% respectively, for triglycerides [20,21].

Amino acid analysis

The plasma was separated from heparinized blood samples by centrifugation at 4°C, and one aliquot was deproteinized with sulfosalicylic acid (10% w/v) as described, and the supernatant was stored along with another aliquot of untreated plasma at −20°C [13]. Individual amino acids were determined on the SSA supernatants by standard procedures using lithium buffer systems on a Beckman 121M Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, Ca.). No specific measures were taken apart from minimizing the period between sampling and analysis, to assure the stability of glutamine, glutamate and aspartate. The values reported reflect this approach to sample handling [16]. Tryptophan was analyzed separately.
and subsequently in trichloracetic acid precipitated plasma and
dialysate by a fluorimetric method [17].

Data are expressed as mean ± SD. Analysis of variance
(One-way ANOVA) with Tukey’s post hoc test was used for
testing the significance of parametric data using SPSS
for windows, version 10.0.1. p < 0.05 was taken as the cut off
value for significance.

Results
Assessment of glycemic, lipid profile and biochemical parameters
in Saudi diabetic patients

In the current study, to evaluate the correlation between glyce-
mic status and amino acid profile in Saudi diabetic patients;
fasting serum glucose level was taken as the primary criterion
of group segregation into hyperglycemic and normoglycemic
patient. Patient demographic criteria were assessed and there
was no significant difference in mean body mass index (BMI)
of different groups, whether among male or female subjects. Serum glucose of 140 mg% was considered as cut-off value
for hyperglycemia according to the institutional guidelines.
Obviously, mean blood glucose was found significantly higher
in hyperglycemic male and female groups (198.3 ± 71.4 and
228.5 ± 61.6 mg%, respectively) compared to their corre-
sponding normoglycemic groups (108.7 ± 14.7 and 110.6
± 22.6 mg%, respectively) (Table 1).

In general, there were no significant differences in lipid profile
parameters between normoglycemic and hyperglycemic
corresponding groups with the exception of serum TG. Serum
TG in male hyperglycemic patients was elevated to about two folds its concentration in normoglycemic male group at $p < 0.05$ (Table 1).

Also, we assessed several muscle (CK), kidney (BUN), and liver (ALP, AST and ALT) biochemical markers in hyperglycemic and normoglycemic Saudi diabetic patients. No significant change in all assessed biochemical parameters was detected with the universal level of significance ($p < 0.05$) (Table 1).

Plasma level of amino acids in Saudi diabetic patients

Concentration of several amino acids of different categories (essential, semi-essential and metabolic indicator amino acids) was assessed in plasma of hyperglycemic and normoglycemic diabetic Saudi patients (males and females). Among essential amino acids, MET showed significant increase in plasma concentration of male hyperglycemic patient ($3.6 \pm 4.4 \mu g/ml$) compared to corresponding normoglycemic group ($1.3 \pm 2.0 \mu g/ml$). PHE showed significant decrease in plasma concentration of female hyperglycemic patient ($1.2 \pm 2.3 \mu g/ml$) compared to corresponding normoglycemic group ($3.4 \pm 4.6 \mu g/ml$) at $p < 0.05$. No significant differences in other essential amino acids between hyperglycemic and normoglycemic diabetic Saudi patients at $P < 0.05$ (males and females) (Table 2).

With respect to semi-essential amino acids, plasma concentrations of both CYS2 and HIS were significantly
decreased in female hyperglycemic Saudi patients (1.0 ± 2.4 and 0.4 ± 0.8 µg/ml, respectively) compared to normoglycemic group (4.2 ± 5.1 and 1.6 ± 2.8 µg/ml, respectively) at p < 0.05. On the other hand, plasma concentration of HIS was significantly increased in male hyperglycemic Saudi patients (2.0 ± 3.0 µg/ml) compared to corresponding normoglycemic (0.6 ± 0.8 µg/ml) group at p < 0.05 (Table 2).

Interestingly, all metabolic indicator amino acids were significantly decreased in hyperglycemic female patients to about 25–40% of its level in the corresponding normoglycemic group (p < 0.05). On the contrary, the plasma levels of all metabolic indicator amino acids in male hyperglycemic patients were higher than the corresponding normoglycemic group, however it did not reach the standard level of significance (p < 0.05) (Table 2).

Fig. 3 Correlation analysis for the association between fasting blood sugar and semi-essential amino acids concentrations in plasma of male Saudi diabetic patients.

Fig. 4 Correlation analysis for the association between fasting blood sugar and semi-essential amino acids concentrations in plasma of female Saudi diabetic patients.
The correlation between plasma level of several amino acids and the glycemic status of diabetic Saudi patients (males and females) was assessed using trend analysis and by calculating the correlation coefficient \( r \). Herein, the absolute value of \( r > 0.5 \) was considered as strong correlation; \( r < 0.25 \) will be described as weak correlation and \( r \) ranging from 0.25 to 0.5 will be considered as moderate correlation. The sign before \( r \) (positive or negative) indicates direct or inverse correlation, respectively. In general, there was clear gender difference in the correlation between plasma concentrations of sugar and all categories of amino acids in Saudi diabetic patients; the plasma level of all amino acids directly increased with the sugar concentration in male Saudi diabetic patients while inversely decreased with the increase in sugar concentration in female Saudi diabetic patients.

There was a positive correlation between all essential amino acids and blood glucose level in male Saudi diabetic patients. The correlation of MET in male diabetic patients with blood glucose was strong \( (r = 0.5) \), while all other essential amino acids showed weak correlation \( (r \) ranging from 0.08 to 0.24) (Fig. 1). On the other hand, MET showed almost no correlation with blood glucose level in female diabetic patients \((r = -0.0014)\). All other amino acids were moderately associated with the plasma level of glucose in female diabetic patients \((r \) ranging from \(-0.33 \) to \(-0.37\)) (Fig. 2).

In male patients, the correlation of all semi-essential amino acids with serum glucose level was positive but weak with \( r \) ranging from 0.02 to 0.17 (Fig. 3). On the other hand, the correlation of semi-essential amino acids with serum glucose in female patients showed some diversity (Fig. 4). High moderate negative correlation \((r = -0.45)\) was observed between serum CYS2 and blood glucose in female diabetic patients. HIS and ARG showed moderate negative correlation with serum glucose with \( r \) ranging from 0.32 to \(-0.37\) in female diabetic Saudi patients. Only TAU showed weak correlation \((r = -0.17)\) with serum glucose in female Saudi diabetic patients.

Similar to semi-essential amino acid, all metabolic indicator amino acids showed positive weak to moderate correlation \((r \) ranging from 0.017 to 0.303) with serum glucose in male Saudi diabetic patients (Fig. 5). In contrast to male Saudi diabetic patients, all amino acids – except ALA – showed moderate to strong correlation co-efficient in female patients (Fig. 6). HCY2 and ORN showed boarder line strong negative correlation with glucose level in female diabetic patients with \( r = 0.5\), respectively. AAA and CIT showed moderate negative correlation.
correlation with glucose level in female patients with $r = -0.44$ and $-0.42$, respectively.

**Discussion**

Diabetes mellitus is major metabolic disease that is associated with several disorders such as, microvascular disorders, hypercholesterolemia, obesity, and other nutritional disorders. The role of amino acids in DM and its metabolic traits have been suggested previously; however, studied to a very limited scale in some populations such as Saudi patients. In the current study, we have assessed the relation between several amino acids of different category and the glycemic status in Saudi diabetic patients. Amino acids assessment can reflect and explain several structural, nutritional, physiological, and biochemical complications of DM; nonetheless in a population with such high prevalence of the disease [1].

Only serum TG in male hyperglycemic patients was elevated in correspondence with the glycemic status. That might be explained by the disturbance in glycolysis associated lipolysis in DM rather than direct relation with plasma glucose level. Also, we cannot ignore nutritional implication that might affect serum TG in Saudi diabetic patients. The elevated TG level in male rather than female diabetic patients might be attributed to hormonal influence [22]. Triglycerides are affected during pregnancy and influenced by sex hormones [23].

The elevated level of MET in male diabetic patient, herein, might be an early indicator for impaired MET metabolism and homocystinuria. Strong correlation between MET and glycemic status in male diabetic patients rather than female might implicate higher susceptibility of male than female Saudi diabetic patients for homocystinuria. In addition, this observation warrants further studies. Microvascular complications of DM such as, coronary artery disease, myocardial infarction, peripheral and cerebral vascular occlusive diseases and stroke have been related to homocystinuria [24,25]. The negative correlation between essential/semi essential amino acid and glycemic status would suggest trend for bad nutritional habits among female rather than in male Saudi patients. In agreement, dietary balance has been an old problem for diabetic patients over decades that warrants careful attention [26,27]. On the other hand, Saudi male diabetic patients would be more prone to diabetic microvascular complication secondary to early signs of homocystinuria [28].

![Fig. 6 Correlation analysis for the association between fasting blood sugar and metabolic indicator amino acids concentrations in plasma of female Saudi diabetic patients.](image-url)
In the current study, TAU did not show any correlation with the glycemic status in male or female patients of Saudi population. Accordingly, no compensatory antioxidant protective machinery would help in the predicted microvascular complications, nonetheless, in male patient with hyperglycemia [29]. Interestingly, the negative correlation between ARG and glucose in female Saudi patients would produce similar vascular occlusive complication due to nitric oxide synthesis impairment. The extensive role of ARG in cardiovascular function, wound healing hormone release (especially insulin and glucagon) and neurotransmission exceeds the synthetic capacity of human body [30].

All metabolic indicator amino acids showed much stronger correlation in female Saudi diabetic patients than in male patients. Taking into consideration the metabolic nature of DM, we can explain such a phenomenon. However, the stronger correlation in female than in male patients would be suggested to be attributed to sex hormone discrepancies [31].

Conclusion

We have shown some correlation between the serum level of several amino acids of different categories and glycemic status among Saudi type-1 diabetic patients in a gender dependent manner. This study and further studies would be required to provide early vision for predicting some metabolic traits in diabetic patients in the Saudi population.

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