Alterations in the levels of plasma amino acids polycystic ovary syndrome

Amal Mofak Saleh
Department of Optics, Al-Noor University College, Iraq, Mosul
Email: Amal.mofak@alnoor.edu.iq

Abstract

Background & objectives: Polycysticovary syndrome (PCOS) is a heterogenous endocrinopathy commonly diagnosed in reproductive age women, predisposing to the development of metabolic disturbances. However, the mechanisms underlying the connection between PCOS, and metabolic disorders are still not well understood. The aim of the study was to investigate amino acid (AA) profile in women with PCOS and to assess its relation with metabolic disturbances. Methods: This study included sixty-five women with PCOS along with a similar number of age-matched normal controls in this study. Levels of 14 amino acids were determined using reverse phase high-performance liquid chromatography. Results The levels of methionine, cysteine, isoleucine, phenylalanine, valine, tyrosine, proline, glycine, lysine and histidine were significantly lower ($p < 0.001$) than in the control group. It was also observed that the levels of arginine and alanine were significantly ($P < 0.001$) higher in the cases compared to the control group. Interpretation & conclusions: Our findings showed significant derangement in the levels of plasma amino acids in women with PCOS which might be due to the oxidative and metabolic stress associated with it. Further studies need to be done to confirm the findings.

Keywords: Amino acids-arginine, branched chain amino acids, cysteine, oxidative stress, polycystic ovary syndrome.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the common endocrinological disorders that affects 4-12 per cent of women during their reproductive years leading to a wide spectrum of clinical manifestations (1). The condition is known to have a major effect throughout life on the metabolic, reproductive and cardiovascular health of affected women. It was first described by Stein and Leventhal as a symptom complex associated with oligomenorrhoea, hirsuitism, obesity and bilaterally enlarged polycystic ovaries(2). The current diagnostic criteria for PCOS is the 2003 Rotterdam European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine (ESHRE/ASRM) revised consensus according to which at least two of the three following criteria are needed for the diagnosis: anovulation or oligoovulation, clinical and/or biochemical signs of hyperandrogenism, and presence of polycystic ovaries on ultrasonography and exclusion of related disorders. Although not enclosed within the diagnostic criteria, a significant feature of PCOS is insulin resistance that results in compensative hyperinsulinaemia, acanthosis nigricans, hyperandrogenism, cardiovascular risk, and type 2 diabetes mellitus (3).

Amino acids function as essential precursors for synthesis of a variety of molecules of significance and also regulate key metabolic pathways and processes that are vital for the proper functioning and maintenance of homeostasis of organisms (4). Besides their role as building blocks of proteins and polypeptides, amino acids are also said to have antioxidant functions. Plasma proteins are considered preventive antioxidants and act by sequestering or inactivating transition metal catalysts (*e.g.*, transferrin, ceruloplasmin). These were also discovered to be chain-breaking antioxidants (5). Normally, the levels of reactive oxygen species (ROS) and antioxidants remain in balance. Oxidative stress occurs when ROS exceeds the level of antioxidants (6). Free radical attack on lipids leads to lipid peroxidation resulting in the formation of reactive aldehydes which can diffuse into the cells, and attack targets far from the site of the original event. Some of these aldehydes have been shown to react with various biomolecules like proteins, DNA, and phospholipid (7).

The role of free radical mediated oxidation of amino acids in PCOS has not been explored (8). Hence, we conducted this pilot study with the aim to find out if there were any changes in the levels of plasma amino acids in women with PCOS (9).

2. Material & Methods

This study included conducting a test on 65 women with PCOS, aged 18-45 years, who attended the outpatient clinics of the Department of Gynecology and Al-Khansa Teaching Hospital in Nineveh Governorate. A similar number of age-matched healthy women with regular menstrual cycles were included as controls. The duration of the study was 6 months from 2021 to 2022. The study was conducted after approval from the Nineveh Health Department

Inclusion criteria: Women (18-45 years old) who had complaints of any of the following conditions: oligomenorrhea/amenorrhea, inability to conceive, hirsutism and polycystic ovary syndrome on ultrasound 3. None of these women received any

hormonal contraceptives or Vitamin supplementation or other important drug therapy. None of them were an alcoholic or a chronic smoker and did not suffer from any other diseases.
Exclusion criteria: Women with the following conditions were excluded from the study: women with hyperprolactinemia, androgen-secreting tumors, congenital adrenal hyperplasia, history of diabetes, liver or pancreatic disease, history of infection or fever in the recent past, and those taking steroids (10).
Measurements were taken for the patients as weight (kg) and height (in metres) to assess body mass index (BMI). Patients were classified as obese and non-obese based on their BMI (those with a BMI greater than 25 kg/m2 were classified as obese and those with a BMI less than 25 kg/m2 as non-obese) (11,12).
Laboratory analysis: Venous blood samples (4 mL) were taken with aseptic precautions in anticoagulant-free places to measure blood hormone (LH), follicle-stimulating hormone (FSH), thyroid stimulating hormone (TSH), and prolactin (13,14). Serum levels of LH (15), FSH (15), TSH (16), and prolactin (15) were estimated. For the purpose of plasma free amino acid measurement, fourteen amino acids were measured—glycine, alanine, valine, leucine, isoleucine, tyrosine, phenylalanine, methionine, cysteine, serine, histidine, arginine, lysine and proline. Venous blood samples (2 ml) were obtained (16,17). The plasma was separated from the samples after centrifugation at 1500 g for 15 min for amino acid determination. Unanalyzed samples were immediately stored at −20 °C and the assay was performed within 2 weeks. Samples were deproteinized with trichloroacetic acid (TCA) and treated with borate and triethylamine (TEA) solution before derivation of the initial column injection. Amino acids were separated using a reverse phase–high performance liquid chromatograph (HPLC) using a German SYKAMN × 4.6 mm apparatus. The particle size is 5 μm. In addition to monitoring the individual amino acids, the amino acids were also grouped as follows: (i) phenylalanine/tyrosine ratio (Phe/Tyr); (ii) glycine: branched chain amino acids (BCAA) ratio (Gly/BCAA); (iii) glycine: valine ratio (Gly/Val), and (iv) BCAA to aromatic amino acids (AAA) ratio- Fischer's ratio(22).

3. Statistical Analysis

For all the continuous variables the results are given in mean ± standard deviation. To compare the means of continuous parameters between groups, those following normal distribution, Student's independent samples t test was performed. To compare the average of continuous parameters between groups, those not following normal distribution, Mann Whitney test was performed.

4. Results

No significant difference was observed between the mean age of patients and controls. A significant elevation of the LH and LH:FSH ratio was observed in patients with PCOS compared to controls. FSH levels decreased significantly ($p < 0.001$). TSH and prolactin levels did not show a significant difference as shown in Table 1.

(Table 1): Baseline characteristics of controls and patients with PCOS

parameters	Controls(n=65)
LH (mLU/ml)	26.43±6.83
fsH(mLU/ml)	3.10±1.26
l.H:fsH ratio	5.8±1.44
TSH (uLU/ml)	0.76±0.43

When compared with the normal controls, the levels of methionine, cystine, isoleucine, phenylalanine, valine, tyrosine, proline, glycine, lysine and histidine were found to be significantly lower in cases. The levels of arginine and alanine were significantly higher in cases than controls. Leucine and serine levels were not significantly different between cases and controls. Glycine/Valine ratio was significantly ($P<0.05$) decreased in cases than controls.

(Table 2): Plasma amino acid levels (μmol/l) in patients with PCOS and controls

Amino acids	Controls (n=65)	Confidence interval (cl)
Valine	172.5±54.1	158.65 ,187.83
Isoleucine	68.54±25.4	63.78, 76.48
Leucine	76.316±33.04	67.98 ,85.80
Phenylalanine	63.38±39.45	56.34, 76.43
Methionine	22.12±17.64	18.22, 26.02
Histidine	65.35±26.54	62.54, 73.17
Lysine	142.55±32.39	126.27, 154.88
Arginine	73.58±18.30	62.04, 76.08
Cystine	31.58±17.86	27.66, 39.47
Serine	82.86±36.38	78.14, 96.64
Proline	196.33±74.44	177.25, 210.7
Tyrosine	72.12±35.12	63.68, 79.56
Glycine	218.43±82.17	197.58, 238.3
Alanine	240.12±119.06	212.35, 268.7

5. Discussion

In our study significantly lower levels of histidine, methionine, cystine, isoleucine, phenylalanine, valine, tyrosine, proline, glycine and lysine were observed in women with PCOS compared with normal controls. Histidine is considered as an anti-inflammatory amino acid and an antioxidant. Being a nucleophilic amino acid, it is vulnerable to modification by lipid peroxidation derived electrophiles such as 2-alkenals, ketoaldehydes and 4-hydroxy-2-alkenals (17). Thus, the low levels of this amino acid found in PCOS patients may be due to its increased utilization as an antioxidant in the presence of oxidative stress. In addition, the increased concentration of free radicals might have led to its oxidation resulting in decreased levels.
Cystine is the oxidized form of cysteine which is a sulphur containing amino acid. Glutathione (GSH) is gamma glutamyl cysteinyl glycine, a tripeptide that is known for its antioxidant property (18). It is synthesized by most cells and is one of the primary cellular antioxidants responsible for maintaining

(19,20) the proper antioxidant state within the body (21,22). Due to the oxidative stress present in patients with PCOS increased amounts of cystine is converted to cysteine and then to GSH to meet the stressful situation. As a result, low levels of cystine are obtained.

Study done by Katayama and Mine (23) revealed that BCAA such as leucine, isoleucine and valine have the capacity to upregulate the activities of the antioxidant enzymes glutathione-S-transferase (GST) and catalase. Thus, the potent induction of catalase and GST may contribute to the protective effects of BCAA against oxidative stress. The low levels of isoleucine and valine obtained in our patients might be because of the increased utilization of these amino acids to combat the oxidative stress. Levels of leucine were similar in cases and controls, the reasons for the same were not known. Due to the increased oxidative stress in patients with PCOS increased amounts of methionine are subjected to its oxidised form resulting in its decreased concentration. Also, because of the high oxidative stress, regeneration of methionine may not be sufficient (24). This might be the reason for low levels of methionine seen in our patients.

Singlet oxygen is an active oxygen species that can cause oxidative damage in biological systems. Proline is an effective quencher of this free radical. It has been shown that proline can reduce the levels of malondialdehyde produced during strong illumination of isolated thylakoids (25). Schuessler and Schilling (26) have proposed that proline residues in polypeptide chains are the site of oxygen radical-mediated cleavage of polypeptide chain. Whether proline acts against other free radicals is not known. Thus, the low levels of proline observed in our study group may be due to the increased oxidative stress leading to increased utilization of proline.

Hyperphenylalaninemia has been associated with conditions like burns (27) and sepsis (28). Tetrahydrobiopterin (BH4) which is the coenzyme for phenylalanine hydroxylase (PAH) is chemically very labile. Hence it is easily susceptible to oxidative damage and thus causes diminution of the PAH activity (29). As a result, the phenylalanine accumulates and tyrosine level decreases. In our study decreased levels of both amino acids were observed; reasons being unknown. Alanine is known to cause induction of glutathione reductase which helps to elevate the levels of GSH (23). This maintains the antioxidant status of the cells. Similarly, arginine through nitric oxide scavenges free radicals and thus functions as an antioxidant (30) In our study elevated levels of alanine and arginine were observed. This could probably be the body's compensatory mechanism to meet the elevated oxidative stress.

Phenylalanine/tyrosine ratio is an indicator of the catabolic state of the body (22). An elevated Phe/Tyr ratio can be seen in conditions like HIV infection (31), phenylketonuria, burns (27), and sepsis (28). In our patients due to the increased oxidative stress an elevated ratio was expected, but no significant difference was seen.

N-acetyl cysteine (NAC) is a derivative of cysteine and is considered as an antioxidant and antiapoptotic agent. Inside the cells it is said to stimulate glutathione production and thus scavenge free radicals. The NAC is also known to influence insulin receptor activity and thus increases the uptake of glucose (32).

In conclusion, significant alterations in the levels of plasma amino acids were observed in patients with PCOS. Whether supplementation of amino acids can restore their levels in plasma or can improve the pathologies in PCOS like infertility, menstrual disturbances and hyperandrogenism has not yet been proved. Hence more studies with a large sample need to be performed to reach a definite conclusion (33).

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