

DECREASED SERUM LEVEL OF GAMMA-AMINO BUTYRIC ACID IN EGYPTIAN INFERTILE FEMALES WITH POLYCYSTIC OVARY SYNDROME IS CORRELATED WITH DYSLIPIDEMIA, TOTAL TESTOSTERONE AND 25(OH) VITAMIN D LEVELS

SMANJEN SERUMSKI NIVO GAMA-AMINO BUTERNE KISELINE KOD NEPLODNIH ŽENA U EGIPTU SA SINDROMOM POLICISTIČNIH JAJNIKA JE U KORELACIJI SA DISLIPIDEMIJOM, UKUPNIM TESTOSTERONOM I 25(OH) VITAMINOM D

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Summary

Background: Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders around the world. Increasing evidence suggests that neurotransmitter Gamma-aminobutyric acid (GABA) is involved in the pathogenesis of PCOS through its central role in the hypothalamus. However, the peripheral role of GABA in PCOS has not been sufficiently investigated in spite of its existence in peripheral organs. First, the aim of this study is to, investigate serum GABA level in Egyptian PCOS patients. Second, to explore the correlation between serum GABA level with Body Mass Index (BMI), dyslipidemia, total testosterone and 25 (OH) vitamin D.

Methods: Eighty PCOS patients and eighty age-matched healthy females were included in this study. All parameters were assessed colourimetrically or with ELISA.

Results: PCOS patients exhibited significantly decreased serum GABA level compared to controls ($p < 0.001$). There

Kratak sadržaj

Uvod: Sindrom policističnih jajnika (PCOS) je jedan od najčešćih endokrinih poremećaja kod žena širom sveta. Sve više dokaza sugerise da je neurotransmiter gamma-amino buterna kiselina (GABA) uključena u patogenezu PCOS na osnovu njene centralne uloge u hipotalamusu. Međutim, periferna uloga GABA u PCOS nije dovoljno istražena uprkos njenom postojanju u perifernim organima. Cilj ove studije jeste da se prvo ispita serumski GABA nivo kod egipatskih PCOS pacijentkinja, a osim toga, potrebno je i istražiti korelaciju između nivoa GABA u serumu i indeksa telesne mase (BMI), dislipidemije, ukupnog testosterona i 25(OH) vitamina D.

Metode: U studiju je uključeno osamdeset pacijentkinja sa PCOS i osamdeset zdravih žena istog životnog doba. Svi parametri su procenjeni kolorimetrijskom ili ELISA metodom.

Rezultati: Kod PCOS pacijentkinja se pokazao značajno sniženi nivo GABA u serumu u poređenju sa kontrolom grupom ($p < 0,001$). Postojala je značajna pozitivna korelacija između

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List of abbreviations: AMH, anti Müllerian hormone; BMI, body mass index, CSF, cerebrospinal fluid; Cyt. p450c: cytochrome p450-c17alpha-hydroxylase; FSH, follicle-stimulating hormone; GABA, gamma-aminobutyric acid; GnRH, gonadotropin-releasing hormone; HDL-C, HDL cholesterol; HC, Hip circumference; LH, luteinizing hormone; IR, insulin resistance; LDL-C, LDL-cholesterol; OS, oxidative stress; PCOS, polycystic ovary syndrome; ROS, reactive oxygen species; TAG, triacylglycerol; TC, total cholesterol; WC, waist circumference

was a significant positive correlation between serum GABA and 25(OH) vitamin D levels ($r = 0.26$, $p = 0.018$), and a significant negative correlation with total testosterone ($r = -0.3$, $p = 0.02$), total cholesterol (TC) ($r = -0.31$, $p = 0.01$) and LDL-Cholesterol (LDL-C) ($r = -0.23$, $p = 0.045$), respectively.

Conclusions: The findings of this study suggest that disrupted GABA level in the peripheral circulation is an additional contributing factor to PCOS manifestations. GABA deficiency was correlated with 25 (OH) vitamin D deficiency, dyslipidemia, and total testosterone. Further investigations for GABA adjustment might provide a promising means for better management of PCOS symptoms.

Keywords: PCOS, GABA, dyslipidemia, 25(OH) vitamin D, neuroendocrine

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder among females of reproductive age (1), with the prevalence of 9–18 % all over the world and is the leading cause of infertility around the globe (1, 2). Moreover, the latest reports showed that PCOS prevalence exceeds 37% in secondary infertile Egyptian females especially in Upper Egypt (3). According to Rotterdam consensus, 2003, PCOS is diagnosed by having at least two of three main criteria; Oligo or anovulation, clinical or biochemical features of hyperandrogenism and polycystic ovaries on ultrasound examination (4). PCOS patients suffered from severe manifestations as infertility, hirsutism, acne, alopecia and disturbed hormonal profile. (5, 6). Moreover, PCOS is highly combined with insulin resistance (IR) (7, 8), dyslipidemia (6, 9) and obesity (10). Heterogenic factors further contribute to PCOS manifestations (11).

PCOS was believed to be a mere ovarian disorder. Nevertheless, increasing basic and clinical research imply that disruption in the neuroendocrine homeostasis of the hypothalamus-pituitary-gonadal axis drives and contributes to PCOS (12, 13). Thereby in PCOS, there is an increased gonadotropin-releasing hormone (GnRH) pulsatile secretion from the GnRH neuron network. This, in turn, causes significant disturbances in luteinizing hormone (LH), follicle-stimulating hormone (FSH) and progesterone levels. Moreover, it decreases hypothalamus sensitivity to progesterone negative feedback regulation. Such disruptions lead to increased androgen synthesis in the theca cells, causing anovulation, ovarian cysts, hirsutism and acne that are prevalent in PCOS (14–16).

Recently, emerging evidence increasingly suggests a significant role for the neurotransmitter Gamma-aminobutyric acid (GABA) in PCOS. Recent animal model studies have shown that elevated androgen levels maintained PCOS by promoting central GABA secretion and activating central GABA-

nivoa GABA i 25(OH) vitamina D u serumu ($r = 0,26$; $p = 0,018$) i značajne negativne korelacije sa ukupnim testosteronom ($r = -0,3$; $p = 0,02$), ukupnim holesterolom (TC) ($r = -0,31$; $p = 0,01$) i LDL holesterolom (LDL - C) ($r = -0,23$; $p = 0,045$), respektivno.

Zaključak: Rezultati ove studije ukazuju na to da je poremećeni nivo GABA u perifernoj cirkulaciji dodatni faktor koji doprinosi manifestacijama PCOS. GABA je povezana sa 25(OH) vitaminom D, dislipidemijom i testosteronom. Dalja istraživanja u vezi sa kontrolom GABA nivoa mogu doprineti boljoj kontroli simptoma PCOS-a.

Ključne reči: PCOS, GABA, dislipidemija, 25(OH) vitamin D, neuroendokrini

ergic receptors stimulation to the neural GnRH cascade (17–19). Furthermore, Kawwass et al. (20) were the first to show that PCOS women exhibited high cerebrospinal fluid (CSF) GABA and total testosterone levels, supporting the central role hypothesis of GABA in PCOS. However, a small sample size, fluctuations in CSF analyses and not assessing serum GABA level were the main limitations in that study.

Interestingly, GABA and GABAergic receptors have been detected in various peripheral tissues as the ovaries, GIT, adrenal medulla and pancreas (21, 22). In the pancreas, adequate blood GABA levels activate GABA A and GABA B receptors, inducing calcium signalling and AKT pathways which promote β islets mass, decrease apoptosis and maintain healthy blood glucose level and proper insulin sensitivity (23). Whereas in the ovaries, GABA acts on the GABAergic receptors to maintain normal progesterone hormone secretion and corpus luteum formation (24) Regarding GIT and fat metabolism, GABA treatment exerts a powerful anti-inflammatory and antioxidant actions that significantly reduce macrophage infiltration in the adipose tissues and inhibit high fat-diet induced obesity (21). Consequently, further investigations are highly required to get a better understanding of GABA level in different biological fluids of PCOS patients.

It was reported that PCOS is characterized by the prevalence of obesity and disturbed levels of triacylglycerol (TAG) and total cholesterol (TC) (24). Interestingly, Ullah et al. (22) recently showed that administration of GABA significantly decreased dyslipidemia in PCOS animal model, which suggests a possible peripheral role for GABA level in PCOS. However, no studies investigated the levels of serum GABA and dyslipidemia in PCOS patients.

Moreover, testosterone, low serum 25(OH) vitamin D are also characteristic features in PCOS (6, 26). It is well documented that 25(OH) vitamin D is a crucial player in follicular development, sensitivity to FSH and Anti-Müllerian hormone (AMH) signalling

(27). Remarkably, Groves et al. (28) found that 25(OH) vitamin D deficiency was associated with a disruption in GABA levels in the brain tissues of adult mice. In line with this, recent reports have shown that 25(OH) vitamin D deficiency could trigger depression through disturbances in neuronal calcium, glutamate and GABA concentrations (29). However, a correlation between GABA and 25(OH) vitamin D levels has not been explored in PCOS patients, either centrally or peripherally. In light of these observations, examining serum GABA levels in PCOS patients can provide a better understanding of its role in PCOS and whether this role is only limited to the CNS or extends to the peripheral organs through different mechanisms. To the best of our knowledge, no previous study examined serum GABA levels in PCOS patients, nor its relation to the biochemical and hormonal findings in PCOS patients.

Hence, we first aimed to investigate serum GABA level in Egyptian PCOS patients compared to healthy controls. Second, the aim was to explore the possible correlation between serum GABA level with body mass index (BMI), dyslipidemia, total testosterone and 25(OH) vitamin D levels in PCOS patients.

Material and Methods

Study Population

The present study included 2 main groups. Group 1 comprised 80 PCOS patients aged from 22 to 29, with primary or secondary infertility as PCOS group. Diagnosis of PCOS was made according to Rotterdam consensus, 2003 (4). Where two out of the three following conditions were required to confirm PCOS diagnosis: Oligo- and/or anovulation, clinical and/or biochemical features of hyperandrogenism (defined by clinical hirsutism (Ferriman-Gallaway score ≥ 6), acne or alopecia and/or elevated androgens) and polycystic ovaries on ultrasound examination. Group 2 comprised 80 apparently healthy females aged from 22 to 30, as the Control group. All control females were apparently healthy, had regular cycles and had neither gynaecological nor endocrinal or neurological disorders.

Subjects having Cushing's syndrome, androgen-secreting tumours, congenital adrenal hyperplasia, diabetes, immunological diseases, any form of malignancy and hyperprolactinemia were excluded from the study. None of the cases included in the study were smokers, nor did they receive oral contraceptive drugs. All subjects had stable body weight for at least 3 months. All subjects were recruited from the outpatient clinics of Al-Azhar University Teaching Hospitals all over Egypt. The study was performed after obtaining informed consent from all subjects and the approval of the ethics committee and the Review Board at Heliopolis University, Cairo, Egypt in accordance with The Code of Ethics of the Declaration of Helsinki.

Sample Preparation

Venous blood samples were collected by venipuncture after 12 hours overnight fasting. Blood was processed within 2 hours after collection and placed in a refrigerator for 1 hour, followed by centrifugation at 2000 g (3000 rpm) for 10 minutes at 4 °C. The supernatant (serum) was separated and stored at -80 °C until analysis.

Laboratory Procedures

Serum GABA level was measured using the Human Gamma-Aminobutyric Acid (GABA) ELISA Kit (MyBioSource, USA). 25-OH Vitamin D (total) level was detected by ELISA kit (DRG Instruments GmbH, Germany). Serum total testosterone was measured by immunoassay (Architect 2nd Generation, Abbott Diagnostics, USA). All procedures were conducted according to the manufacturer's protocol. Serum TAG, TC and HDL-cholesterol (HDL-C) were determined using colourimetric enzymatic assay (Diamond Diagnostics, D-P international, Egypt). LDL-cholesterol (LDL-C) level was calculated using the Friedewalds formula:

$$\text{LDL} = \text{TC} - [(\text{TAG}/5) + \text{HDL-C}] \quad (30).$$

Anthropometric Measurements

Anthropometric measurements included body weight, height, mid-upper arm, thigh waist and hip circumferences and abdominal skinfold thickness. All measurements were taken 3 times on the left side of the body. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with patients standing with their backs leaning against the stadiometer of the same scale. BMI was calculated as weight in kilograms divided by height in square meters (kg/m^2). Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic, non-stretchable tailor's tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Skin-fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) when it was taken to the nearest 0.5 mm. Abdominal skin fold was at 5 cm adjacent to the umbilicus to the right side. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program.

Statistical Analysis

Differences in variables between the two groups of the study were assessed using Mann Whitney's test. Continuous data was presented as a median and interquartile range. Spearman's Correlation was done to detect the presence, degree and direction of the association between the measured variables in PCOS patients. For all data, p value of < 0.05 (two-tailed) was considered significant. Statistical analysis were performed using the SPSS package (version 23 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Biochemical and hormonal data in PCOS and Controls

Patients with PCOS and normal controls did not differ significantly in age. PCOS patients showed significantly higher BMI, serum TAG, TC and LDL-C levels compared to healthy controls ($p < 0.001$) whereas serum HDL-C was significantly lower in PCOS in comparison to controls ($p < 0.001$). A detailed description of the demographic and biochemical characteristics of both groups are shown in Table I.

PCOS group showed significantly lower GABA levels and significantly higher serum total testosterone levels compared to healthy controls ($p < 0.001$). Moreover, PCOS had significantly lower serum 25(OH) vitamin D level compared to the control group ($p < 0.001$). Detailed data on serum GABA and hormonal profile are shown in Table II.

Table I Demographic and biochemical characteristics of PCOS group vs. normal controls. Data are presented as a median and interquartile range.

Parameters	PCOS N = 80	Control N = 80	p Value
Age (years)	26 (25–27)	25 (24–27)	0.9
BMI kg/m ²	26.3 (24–28.3)	23.33 (22.5–25.8)	< 0.001*
TAG mmol/L	2.56 (2.37–2.8)	1.4 (1.32–1.54)	< 0.001*
TC mmol/L	5.45 (5.21–5.7)	4.1 (3.9–4.23)	< 0.001*
LDL-C mmol/L	3.83 (3.59–4.06)	2.2 (1.97–2.33)	< 0.001*
HDL-C mmol/L	1.13 (1–1.23)	1.7 (1.5–1.73)	< 0.001*

*indicates significant difference at p value < 0.05.

Table II Serum GABA level, testosterone and 25(OH) vitamin D of PCOS group vs. normal controls. Data are presented as median and interquartile range.

Parameters	PCOS N=80	Controls N=80	p Value
GABA (μ mol/L)	1.95 (1.3–2.57)	4.3 (3.7–5.25)	< 0.001*
Testosterone (nmol/L)	4.7 (3.9–4.9)	0.6 (0.42–0.7)	< 0.001*
Vitamin D (nmol/L)	38.5 (33.51–43.75)	77.5 (63.05–90.2)	< 0.001*

*indicates significant difference at p value < 0.05.

Correlation between different biochemical and hormonal data in PCOS group

The correlation was investigated between different measured parameters in PCOS group and serum GABA levels. There was a statistically significant positive correlation between serum GABA and 25(OH) vitamin D levels ($r = 0.26$, $p = 0.018$) and a statistically significant negative correlation with serum total testosterone level ($r = -0.3$, $p = 0.02$). Moreover, GABA level was significantly negatively correlated with TC ($r = -0.31$, $p = 0.01$) and LDL-C ($r = -0.23$, $p = 0.045$) levels, respectively. However, there was no statistically significant correlation with BMI, TAG and HDL-C.

Discussion

In the present study, we aimed to investigate serum GABA level in Egyptian PCOS patients and explore its relation to BMI and biochemical characteristics of PCOS patients. PCOS patients showed significantly higher BMI, TAG, TC, LDL-C levels and significantly lower HDL-C levels compared to age-matched healthy females. Such findings agree with the previously documented findings of PCOS (6, 31–33). Moreover, dyslipidemia is highly prevalent and reported even in lean PCOS females (34).

Serum GABA level was significantly decreased in PCOS subjects compared to healthy controls. However, Kawwass et al. (20) observed significant high levels of GABA in the CSF of PCOS women compared to healthy controls. Additionally, patients who take valproate antiepileptic drugs to induce GABAergic tone in the brain, usually show PCOS like symptoms (35, 36). It is worth mentioning here that the inverted level of neurotransmitters and biomarkers between CSF and other biological fluids is a well-known phenomenon. It can be explained by differences in neuronal uptake, changes in production, metabolism and clearance in different tissues, which lead to accumulation in the brain and CSF while decreasing in plasma and serum (37).

In the present study, there was a statistically significant negative correlation between serum GABA and total testosterone levels. This goes in line with a recent rat model study, where oral GABA intake had an obvious anti-androgenic effect that was demonstrated by significantly decreasing serum total testosterone level and reduced formation of ovarian cysts (22). Furthermore, it has been documented that IR causes hyperandrogenism by stimulating the enzyme cytochrome p450-c17alpha-hydroxylase (cyt. p450c). Any slight disturbance in the factors responsible for the up and down-regulation of cyt. p450c in theca cells, may cause ovarian hyperandrogenism despite normal LH secretion (38, 39). Moreover, IR potentiates the effects of LH on theca-interstitial cells, resulting in increased androgen production while arresting the follicular maturation process which leads to PCOS (40, 41). On the other hand, treatment with GABA is reported to improve insulin sensitivity through its peripheral receptors in the pancreas, ovaries and adrenal medulla in animal models (21, 42). Consequently, this might reduce androgen synthesis by the ovarian theca cells, as IR is a critical contributing factor in excess androgen secretion (43).

Recently, oxidative stress (OS) has been documented in infertile women with PCOS (44–46). It has been demonstrated to be directly correlated with total testosterone and androstenedione which may, as a result, contribute to hyperandrogenism in PCOS women. Furthermore, OS is involved in altered steroidogenesis in the ovaries, thus contributing to increased androgen production, disturbed follicular development and, ultimately infertility (47, 48). Moreover, it has been shown that OS correlates with IR (48). Interestingly, it was reported that GABA recovered the activities of several antioxidant enzymes in letrozole induced PCOS model (22). Furthermore, GABA has been reported as a positive regulator of antioxidant enzymes as it reduces reactive oxygen species (ROS), so it maintains tissue protection, regeneration in addition to normoglycemia in humans and animals. Hence, counteracts both OS and IR (23, 50, 51).

Interestingly, we found a significant positive correlation between serum GABA and 25(OH) vitamin D levels in PCOS patients. Recent studies are increasingly pointing out the possible link between 25(OH) vitamin D and GABA. Groves et al. (28) reported that 25(OH) vitamin D deficient mice showed disruptions in GABA level and GABA synthesizing enzymes in the brain tissues, leading to

behavioural and neurochemical alterations. Additionally, deficiency of Vitamin D has been linked to poor GABA production and blood levels in humans (52). The findings of our study support this hypothesis and imply the possible role of both GABA and 25(OH) vitamin D alterations in PCOS manifestations both peripherally and centrally. Moreover, disrupted levels of both GABA and 25(OH) vitamin D levels in PCOS, can contribute to the impaired emotional and cognitive issues prevalent among PCOS patients (29, 53). Further investigations are needed to confirm this assumption.

In the current investigation, a significant negative correlation between serum GABA level and TC and LDL-C levels. There is a strong association between dyslipidemia observed in PCOS and IR (8, 25). Meanwhile, recent studies reported that pancreatic islets secrete GABA which has multiple effects on β cells through increasing insulin secretion, stimulating cell proliferation and tissue regeneration (54, 55). In line with these studies, GABA intake was reported to significantly improve the lipid profile of letrozole-induced PCOS rats (22). Moreover, GABA has been reported to be involved in reducing TC in humans (50, 51). Furthermore, GABA is reported to maintain adequate lipid profile in humans and animals (23, 51). Such an effect may be attributed to the protective role of GABA on glucose homeostasis and antioxidant enzymes, improving overall insulin sensitivity (22).

In conclusion, Egyptian PCOS patients showed significantly low serum GABA levels compared to healthy controls. This impairment was significantly correlated with dyslipidemia and 25(OH) vitamin D deficiency and serum total testosterone level. The findings of the present study strongly suggest that the disrupted GABA level in the peripheral circulation of PCOS patients contribute to PCOS manifestations, mostly through lack of the ability of improvement of IR and its deficient antioxidant potential. Further investigations of serum GABA antioxidant potential together with IR on different populations can provide a promising means for better management of PCOS symptoms.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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