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Hormone Imbalance in Women with Infertility Caused by Polycystic Ovary Syndrome: Is There a Connection with Body Mass Index?

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Abstract

BACKGROUND: The most common cause of female infertility is ovulation disorders, and the most common non-ovulatory cause is polycystic ovary syndrome (PCOS).

AIM: The aim of the study was to define the reproductive hormone levels in women with infertility due to PCOS, depending on the body mass index (BMI).

PATIENTS AND METHODS: The present study involved 100 women aged 25–39 years with infertility due to PCOS (PCOS group) and 30 women of the same age with infertility due to tubal-peritoneal causes (control group). Infertility due to PCOS was diagnosed according to the Rotterdam criteria. Hormone levels (anti-Müllerian [AMH], follicle-stimulating [FSH], luteinizing [LH], prolactin, estradiol, and testosterone) in blood serum were determined by ELISA.

RESULTS: We detected a correlation between BMI and sex hormone levels as well as LH/FSH ratio. Notably, the ratio of LH/FSH in women with PCOS was significantly different compared to the control group, while at the same time, PCOS was significantly more frequent in overweight and obese patients compared to those with normal BMI. For instance, the LH/FSH ratio was 30.35% higher in women with Class 2 obesity than in the group of women with normal weight. However, in women with both PCOS and Class 3 obesity, the LH/FSH ratio was the lowest among those with a BMI of 25.0-39.9.

CONCLUSIONS: We found a hormonal imbalance in women with infertility caused by PCOS: Increased levels of AMH and LH, estradiol, and testosterone and decreased FSH levels. Analysis of the relationship between the concentration of reproductive hormones and BMI showed a weak inverse relationship between BMI with FSH levels, as well as a direct correlation with the levels of LH, prolactin, estradiol, and testosterone, and LH/FSH ratio. Thus, obesity exacerbates the hormonal imbalance in women with infertility caused by PCOS.

Introduction

Infertility is a common disorder that has a considerable socioeconomic impact on populations while deeply affecting personal health and quality of life of individuals [1]. In Ukraine, according to the national Ministry of Health 2002–2012 data, 12.5 thousand women were diagnosed with infertility each year (122.8 ± 0.33/100,000 women) [2]. Conditions such as ovulatory disorders, endometriosis, chromosomal abnormalities, fallopian tubal disease, unexplained infertility, and sperm-related factors can all result in infertility [3], [4]. The reproductive system of both men and women can be affected by untreated sexually transmitted infections, with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* being the most common STIs resulting in infertility [5], [6].

However, the most common cause of female infertility is ovulation disorders, and the most common non-ovulatory cause is polycystic ovary syndrome (PCOS) [1]. PCOS is a complex hormonal and metabolic disorder characterized by oligomenorrhea or amenorrhea, hyperandrogenism, and infertility [7]. According to Joham *et al*., the prevalence of PCOS varies from 6% to 21%, depending on the diagnostic criteria and studied population [8]. Thus, using the diagnostic criteria proposed by the National Institute of Child Health and Human Development (NICHD, USA) and the American National Institute of Health (NIH, USA), the prevalence of PCOS is 4–8% among women of reproductive age, while according to the Rotterdam criteria, about 18% of women have PCOS [9].

The main pathophysiological components of PCOS are gonadotropic dysfunction and insulin resistance, which are often associated with high body mass index (BMI) [10]. Overweight or obesity is diagnosed in approximately 60–80% of PCOS patients [11]. Although many obese women are fertile, high BMI contributes to an increased risk of fertility disorders. Obesity is a recognized cause of menstrual and ovulation disorders and infertility [12]. Since obesity exacerbates the hormonal and clinical features of PCOS and women suffering from PCOS have a high risk of obesity [13], we suggest that hormonal imbalance resulting in certain body features can be linked to the pathogenesis of PCOS.

The aim of the study was to define the reproductive hormone levels in women with infertility due to PCOS, depending on the BMI.

Materials and Methods

The study involved 100 women aged 25–39 years with infertility due to PCOS (PCOS group) and 30 women of the same age with infertility due to tubal-peritoneal causes (control group), who were treated at "The Clinic of Professor Stefan Khmil" medical center from October 2015 to March 2019. The study protocol was approved by the Medical Ethics Committees of I. Horbachevsky Ternopil National Medical University (No. 34-25/10/2019) and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from all patients.

Infertility due to PCOS was diagnosed according to the Rotterdam criteria [9]: Menstrual dysfunction (presence of oligoovulation, anovulation resulting in amenorrhea, oligomenorrhea, and opsomenorrhea) or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries confirmed by ultrasound. The presence of at least two of the three manifestations results in PCOS diagnosis. The retrospective analysis group did not include patients with adenomyosis, genital endometriosis, and uterine fibromyoma (submucous, subserous, or intramural types). The cases of tubal-peritoneal infertility were diagnosed by laparoscopy or hysterosalpingography.

The height and body weight were measured using standard procedures with a stadiometer and scales, respectively. WC was measured between the ridges of iliac bones and the edge of rib arches at the end of exhalation in normal breathing. HC was measured at the point of maximum buttocks circumference in a horizontal position. BMI was calculated using the formula BMI = Body weight (kg)/height (m^2). The data were interpreted according to the WHO recommendations: Normal weight in the range of 18.0–24.9 kg/m^2; overweight (pre-obesity), 25.0–29.9 kg/m^2; Class 1 obesity, 30.0–34.9 kg/m^2; Class 2 obesity, 35.0–39.9 kg/m^2; and Class 3 obesity, >40 kg/m^2 [14].

The levels of gonadotropin and sex hormones were determined by the laboratory of "The Clinic of Professor Stefan Khmil" medical center (accreditation number 268604/2019). Basal levels of follicle-stimulating hormone (FSH) (catalog number EIA-1288), luteinizing hormone (LH) (catalog number EIA-1289), estradiol (catalog number EIA-4399), general testosterone (catalog number EIA-1559), anti-Müllerian hormone (AMH) (catalog number DSL-10-14400), and prolactin (catalog number EIA-1291) were determined on days 1–3 and progesterone levels on days 21–22 of the menstrual cycle. Hormone levels in blood serum were determined by ELISA using the "Diagnostic Systems Laboratories, Inc." test systems (USA) and "DRG Diagnostics" (Germany).

Statistical analysis of the results was carried out using Microsoft Office Excel and Statistica 7.0 software. Normality of distributions in each of the study groups was determined using nomograms, Shapiro–Wilk, and Lilliefors tests. For the groups with values obeying with normal distribution, central tendency values were expressed as a mean ± SD. For the groups with values not following the normal distribution, central tendency was expressed as a median (M), followed by lower and upper quartiles (LQ; UQ). To determine the impact of each factor on infertility, we constructed frequency tables and performed Fisher's exact test. Probability $p < 0.05$ was considered as significant. The analysis of three more normally distributed variables was carried out using ANOVA followed by the Tukey's range test for subsequent pairwise comparison of groups.

Results

In women with infertility due to PCOS, the levels of anti-Müllerian and luteinizing hormones, estradiol, and testosterone were elevated compared to the control group. At the same time, the concentration of FSH was significantly reduced (by 35.9%) (Table 1). The LH/FSH ratio used in clinical practice is used, was significantly higher (1.5 times) in the patients with PCOS compared to the control group. This indicates a pronounced ovulatory reserve and is a risk factor for ovarian hyperstimulation syndrome [15].

Table 1: Sex hormone levels and LH/FSH ratio in patients with infertility caused by PCOS, M (LQ; UQ)

Hormones	Control group	PCOS group	Mann–Whitney confidence interval, p
AMH, nmol/L	2.10 (1.80; 2.30)	6.85 (5.30; 8.70)	<0.001*
FSH, IU/L (days 2–3 of MC)	8.70 (8.40; 9.10)	6.40 (6.10; 7.15)	<0.001*
LH, IU/L (days 2–3 of MC)	7.25 (7.00; 7.70)	8.35 (7.40; 9,40)	<0.001*
LH/FSH	0.81 (0.80; 0.90)	1.19 (1.09; 1.36)	<0.001*
Prolactin, μg/L (days 2–3 of MC)	13.05 (12.40; 14.00)	12.50 (10.95; 13.95)	>0.05
Estradiol, pg/L (days 2–3 of MC)	27.20 (26.,80; 28.40)	28.10 (27.30; 32.40)	<0.05*
Progesterone, ng/L (days 21–23 of MC)	14, 15 (13.50; 15.30)	13.85 (12.80; 15.15)	>0.05
Testosterone, nmol/L (days 2–3 of MC)	1.40 (1.30; 1.80)	3.70 (1.95; 4.75)	<0.001*

*Statistically significant results. LH: Luteinizing, FSH: Follicle-stimulating, AMH: Anti-Müllerian, PCOS: Polycystic ovary syndrome, LQ; UQ: Lower and upper quartiles.

In this study, 42 patients had normal weight (95% CI [32.79; 51.80]), and 58 were overweight or obese to various classes: 0 (overweight) −24 % (95% CI [16.64; 33.29]), Class 1 obesity −16 % (95% CI [9.99; 24.53]), Class 2 obesity −13 % (95% CI [7.62; 21.12]), and Class 3 obesity −5 % (95% CI [1.87; 11.46]). We detected a correlation between BMI and sex hormone levels (FSH, prolactin, estradiol, and testosterone) as well as LH/FSH ratio (Table 2). Notably, the ratio of LH/FSH in women with PCOS was significantly different compared to the control group, while at the same time, PCOS was significantly more frequent in overweight

Table 2: The levels of reproductive hormones and the LH/FSH ratio depending on BMI in women with infertility due to PCOS (M (LQ; UQ)

Groups	1	2	3	4	5
Indicators	Normal body weight	Overweight	Class 1 obesity	Class 2 obesity	Class 3 obesity
AMH	6.75 (5.40; 8.30)	7.30 (5.80; 10.30)	6.50 (4.95; 8.35)	6.40 (6.10; 10.10)	6.20 (5.00; 6.30)
FSH	6.80 (6.20; 8.00)	6.40 (6.20; 6.90)	6.45 (6.20; 7.10)	6.00 (6.00; 6.10)	6.20 (6.00; 6.30)
LH	7.85 (7.00; 9.10)	8.60 (7.95; 9.45)	8.90 (7.85; 10.85)	9.00 (8.20; 9.70)	8.30 (7.90; 11.70)
LH/FSH	1.12 (1.08; 1.16)	1.27 (1.17; 1.39)	1.27 (1.15; 1.44)	1.46 (1.34; 1.56)	1.08 (1.08; 1.31)
Prolactin	11.55 (7.00; 12.80)	12.75 (12.00; 13.75)	16.30 (12.50; 18.35)	12.40 (12.20; 12.70)	14.50 (14.10; 14.50)
Estradiol	27.85 (26.70; 29.30)	28.40 (27.10; 29.30)	28.95 (28.35; 34.70)	35.60 (28.40; 36.30)	35.80 (34.20; 35.90)
Progesterone (day 21–23 of MC)	13.95 (12.80; 15.20)	13.75 (12.75; 14.20)	13.55 (12.70; 14.70)	14.50 (12.80; 15.30)	15.20 (14.10; 15.20)
Testosterone	1.95 (0.90; 3.00)	4.10 (3.50;4.70)	4.25 (3.65; 6.20)	5.20 (4.30; 6.80)	5.10 (5.00; 7.30)

p>0.05 for Kruskal–Wallis test for AMH, LH, progesterone (days 21–23 of MC). FSH: p<0.001 for Kruskal–Wallis test; p<0.005 (using Bonferroni correction) for pairwise comparisons of groups 1–4, 2–4, 3–4. LH/FSH: p<0.001 for Kruskal–Wallis test; p<0.005 (using Bonferroni correction) for pairwise comparisons of groups 1–2, 1–3, 1–4. Prolactin: p<0.05 for Kruskal–Wallis test; p<0.005 (using Bonferroni correction) for pairwise comparisons of groups 1–3, 1–5. Estradiol: p<0.05 for Kruskal–Wallis test; p<0.005 (using Bonferroni correction) for pairwise comparisons of groups 1–3, 1–4, 1–5, 2–5. Testosterone: p<0.001 for Kruskal–Wallis test; p<0.005 (using Bonferroni correction) for pairwise comparisons of groups 1–2, 1–3, 1–4, 1–5. LH: Luteinizing, FSH: Follicle-stimulating, AMH: Anti-Müllerian, PCOS: Polycystic ovary syndrome, LQ; UQ: Lower and upper quartiles.

and obese patients compared to those with a BMI in the range of 18.5–24.9. For instance, the LH/FSH ratio was 30.35% higher in women with Class 2 obesity than in the group of women with normal weight. However, in women with both PCOS and Class 3 obesity, the LH/FSH ratio was the lowest among those with a BMI of 25.0–39.9.

When comparing the level of hormones in patients with infertility caused by PCOS to those with tubal-peritoneal infertility (control group), the decrease in FSH levels was a more important contributor to the high than the increase in LH. Similarly, in women with a BMI of more than 25.0, the change in LH/FSH ratio was mainly driven by the change in FSH (Table 2). Thus, the lowest level of FSH was found in women with infertility due to PCOS and Class 2 obesity, an 11.76% decrease compared to the group of women with normal weight.

The levels of prolactin in the blood of women with infertility caused by PCOS and Classes 1 and 3 obesity were also significantly higher than in the group of women with normal weight. Hyperprolactinemia in PCOS is most often associated with increased levels of metabolic estrogens. Estradiol levels in the blood serum were significantly higher in obese women with infertility caused by PCOS compared to women with normal weight. Thus, estradiol levels in women with PCOS and Class 3 obesity were 28.55% higher compared to women with normal body weight and 26.06% compared to overweight women (Table 2).

Testosterone levels in women with infertility caused by PCOS and with a BMI of more than 25.0 kg/m^2 were significantly higher compared to the group of women with normal body weight. However, this marker did not demonstrate significant differences in women with different degrees of overweight and obesity (Table 2).

We studied the relationship between BMI and reproductive hormone levels in the patients with PCOS caused infertility. BMI had a weak inverse correlation with FSH levels ($r = -0.28$, $p < 0.05$), as well as direct correlation with the LH/FSH ratio ($r = 0.40$, $p < 0.05$) and the levels of LH ($r = 0.25$, $p < 0.05$), prolactin ($r = 0.32$, $p < 0.05$), estradiol ($r = 0.43$, $p < 0.05$), and testosterone ($r = 0.68$, $p < 0.05$).

Discussion

Since obesity exacerbates the hormonal and clinical features of PCOS and women suffering from PCOS have a high risk of obesity [13], we analyzed the level of hormones relative to BMI in women with infertility due to PCOS. In our study, 42% of women with infertility caused by PCOS had normal body weight. According to Gambineri *et al*., only 33–50% of women with PCOS are overweight or obese, so obesity is not the only factor affecting the prevalence or severity of PCOS [16]. On the one hand, obese women have a higher risk of developing PCOS [17]. At the same time, women with PCOS have a higher risk of developing obesity [18]. Therefore, it remains unclear whether obesity leads to PCOS, or, conversely, PCOS leads to obesity.

AMH is a glycoprotein that is synthesized by the granular cells of small antral and periantral ovarian follicles. Serum AMH levels strongly correlate with the number of antral follicles and are more strongly associated with ovarian reserve than FSH or estradiol [19]. Our findings indicate that serum AMH levels in women with PCOS increase, corroborating results of other studies [20]. This is probably due to an increase in the number of small antral follicles in PCOS. Diwailly argues that serum AMH levels are one of the main markers of PCOS [21]. Any of the factors that disrupt the functioning of granular cells, including obesity, can affect the production of AMH [22], [23]. The relationship between serum AMH and BMI still remains unclear. Some researchers found a weak negative correlation between serum AMH concentration and BMI in women with PCOS [24], [25], but, after correcting for age, this relationship disappeared. Our results are consistent with no correlation between AMH and BMI [26], [27]. It is worth noting that scientists who found a negligible effect of metabolic status on serum AMH levels in PCOS do not recommend considering BMI when interpreting AMH levels in clinical practice [28]. Changes in serum AMH in women with PCOS are mainly associated with impaired production of gonadotropins and steroid hormones. We found a significant decrease in FSH and an increase in LH levels in obese patients with PCOS. There is also a weak inverse relationship between BMI and FSH and weak direct relationship between BMI and LH levels. These

data can be confirmed by a significantly higher ratio of LH/FSH in patients with PCOS. Literature presents controversial conclusions on the changes to LH, FSH, and LH/FSH ratio in PCOS and obesity. Banaszewska *et al*. did not find significant differences between LH/FSH ratio means in the groups of women with and without PCOS [29]. Another study found elevated LH/FSH ratio in patients with PCOS, notably with no correlation to age and BMI [30]. A study by Esmaeilzadeh *et al*. showed that specific age (≥35 years), BMI (≥25 kg/m^2), and acne were significant indicators of metabolic disorders (including obesity) in women with PCOS [31]. Studies confirm the relationship between LH, weight, and PCOS: Insler *et al*. reported that in women with PCOS and normal body weight, blood serum levels were significantly higher compared to patients with obesity, while Yanira *et al*. found an inverse correlation between LH and BMI in women with PCOS [32], [33]. A study by Alnakash and Al-Tae'e showed an inverse relationship between FSH and BMI in obese women with PCOS [34], which our study confirms. The pathological secretion of gonadotropin in PCOS results in elevated serum LH levels and increased LH/FSH ratio [35]. An increase in gonadotropin-releasing hormone upregulates transcription of the LH β-subunit through the FSH β-subunit, which leads to an increase in the LH/FSH ratio in PCOS patients [36].

This study found that in patients with PCOS, testosterone levels significantly increase ($p < 0.001$). At the same time, testosterone levels are the lowest in PCOS patients with normal body weight compared to overweight/obesity ($p < 0.05$), and they significantly correlate with the type of obesity [37]. Nevertheless, the findings on the effect of obesity on the production of androgens in blood serum in PCOS are contradictory. Moran *et al*. report that testosterone and androstenedione levels in PCOS patients are not connected with BMI [11]. However, obesity produces a decrease in sexual hormone-binding globulin, increasing free androgens levels [38]. Another study indicates that obesity causes an increase in testosterone levels in PCOS patients [39], which confirms the results of our study. There is a broad agreement that women with PCOS experience an increase in visceral and subcutaneous fat accumulation caused by an increased rate of androgen production [40]. Furthermore, Escobar-Morreale *et al*. suggest that genetic or induced excess of testosterone may be the main cause of obesity-associated with PCOS [41]. On the other hand, Balen *et al*. found the same metabolic disorders in women with PCOS and normal body weight as in obese patients [42].

The data on the causative relationship between hyperprolactinemia and PCOS remain inconclusive. Some authors believe that hyperprolactinemia in PCOS is associated with hyperestrogenism and steroidogenesis disorders, while others consider hyperprolactinemia to be one of the factors that underlie the pathogenesis of PCOS [43], [44]. The apparent connection of hyperprolactinemia with PCOS can be explained by high LH levels in women with PCOS, leading to a secondary decrease in dopaminergic tone, causing elevated prolactin levels. Another model proposes that increased prolactin secretion under PCOS is the effect of hyperestrogenemia [45]. Finally, a study by Delcour *et al*., as well as our data, does not show any connection between the prolactin levels and PCOS [44]. However, there a link between obesity and pathogenesis of hyperprolactinemia: In obese women, the spontaneous release of prolactin was significantly intensified and correlated with the size of visceral adipose tissue [46]. Studies also suggest that decreased circulating leptin/estrogen levels can reduce prolactin levels. Our results underscore the correlation between prolactin levels and BMI, with significantly higher prolactin levels found in women with the android type of obesity. However, it remains unclear whether hyperprolactinemia association with the weight gain is due to the stimulation of lipogenesis or due to dysregulation of the dopaminergic tone of the central nervous system [47].

Our study found a small but significant increase in serum estradiol levels in PCOS patients. The literature on the levels of estradiol in PCOS is inconclusive. Anovulation is associated with low estradiol secretion, mainly due to peripheral extraglandular conversion and minimal progesterone production [48]. Hashemi *et al*. found decreased estradiol levels in PCOS, while studies point out to exceedingly high estradiol levels [49], [50]. The increase in the level of bioavailable estradiol in PCOS can be attributed to reduced sex hormone-related globulin levels [51], which are also associated with obesity and testosterone levels [38]. Estrogens known to normalize body weight and glucose homeostasis, preventing obesity [52]. Although high levels of estradiol can prevent the development of obesity, traditionally, obesity characterized by relative hyperestrogenemia [53]. In our study, the level of estradiol correlated with BMI. These results suggest estradiol-mediated dysregulation of adipose tissue formation or a decrease in sensitivity to estradiol.

There are some limitations in our study, the most important is relatively small sample size. This can affect the significance of some results, and we suggest that additional research is needed to confirm our findings.

In our study, hormone levels were measured by ELISA method. However, liquid chromatography/mass spectrometry (LC/MS) is considered a working standard for sex steroid assay because of its high sensitivity and ability to detect even the lowest concentrations of the hormones.

Conclusions

We found a hormonal imbalance in women with infertility caused by PCOS: increased levels of

anti-Müllerian and luteinizing hormones, estradiol, and testosterone and decreased FSH levels. Analysis of the relationship between the concentration of reproductive hormones and BMI showed a weak inverse relationship between BMI with FSH levels, as well as a direct correlation with the levels of LH, prolactin, estradiol, and testosterone, and LH/FSH ratio. Thus, obesity exacerbates the hormonal imbalance in women with infertility caused by PCOS.

Data Availability

The data of this study are available by request.

Authors' Contributions

Stefan Khmil contributed to study design, Mariya Khmil conducted research, data, and statistical analysis, and Mariya Marushchak supported in interpretation of findings and drafting of the manuscript.

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