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ARAŞTIRMA / RESEARCH

Progesterone and progesterone-induced blocking factor (PIBF) levels in non-obese women with polycystic ovary syndrome

Polikistik over sendromlu obez olmayan kadınlarda progesteron ve progesteron kaynaklı bloke edici faktör (PIBF) düzeyleri

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Abstract

Purpose: This study aimed to evaluate the level of progesterone and progesterone-induced blocking factor (PIBF), an immune mediator, in non-obese patients with polycystic ovary syndrome (PCOS).

Materials and Methods: Totally 72 patients were recruited into study and divided into 2 groups: The first group was patients diagnosed with PCOS (n = 36) and the second was the healthy control group (n=36). The diagnosis of PCOS was made according to Rotterdam diagnostic criteria. All patients were 18–35 years old and non-obese (body mass index (BMI) < 25 kg/m2). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), prolactin (PRL), total testosterone, and dehydroepiandrostenedione sulfate (DHEA-S) levels were measured on the third day of the menstrual cycle. On the 21st day of the same menstrual period, fasting blood glucose, insulin, progesterone, and PIBF levels were measured.

Results: Demographic and clinical characteristics of study participants were similar between the two groups. Serum FSH, E2, TSH, PRL, DHEA-S, total testosterone, fasting blood glucose, fasting insulin, homeostatic model assessment for insulin resistance (HOMA-IR), and hemoglobin A1c values were similar between the groups. Differences in LH, LH/FSH ratio, serum progesterone, and serum PIBF were statistically significant.

Conclusion: Progesterone and PIBF levels decreased in non-obese PCOS patients. We suggest that even in the absence of obesity, which is the origin and enhancer of

Öz

Amaç: Bu çalışmanın amacı obez olmayan polikistik over sendromlu (PKOS) hastalarda progesteron ve bir immün mediatör olan progesteron kaynaklı bloke edici faktör (PIBF) düzeylerini değerlendirmektir.

Gereç ve Yöntem: Hastalar iki gruba ayrıldı. Birinci grup PKOS tanısı alan hastalar (n=36), ikincisi sağlıklı kontrol grubu (n=36) idi. Rotterdam tanı kriterlerine göre PKOS tanısı konuldu. Hastalar 18-35 yaşları arasındaydı, obez değildi (Vücut kitle indeksi (VKİ)<25 kg/m2). Menstrual siklusun üçüncü gününde folikül uyarıcı hormon (FSH), luteinize edici hormon (LH), östradiol (E2), tiroid uyarıcı hormon (TSH), prolaktin (PRL), total testosteron ve dehidroepiandrostenedion sülfat (DHEA-S) seviyeleri ölçüldü. Aynı siklusun 21. gününde açlık kan şekeri, insülin, progesteron ve PIBF düzeyleri ölçüldü.

Bulgular: Çalışma gruplar arasında demografik ve klinik özellikleri benzerdi. Serum FSH, E2, TSH, PRL, DHEA-S, toplam testosteron, açlık kan şekeri, açlık insülini, insülin direnci için homeostatik model değerlendirmesi (HOMA-IR) ve hemoglobin A1c değerleri gruplar arasında benzerdi. LH, LH/FSH oranı, serum progesteron ve serum PIBF arasındaki farklar istatistiksel olarak anlamlıydı.

Sonuç: Sonuçlarımız obez olmayan PKOS hastalarında progesteron ve PIBF düzeylerinin düştüğünü gösterdi. PKOS'ta inflamasyonun kaynağı olan ve onu alevlendiren obezitenin yokluğunda bile, altta yatan immünomodülatör olarak düşük PIBF'nin komplikasyonları tetikleyeceğini öneriyoruz.

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inflammation in PCOS, low PIBF as the underlying immunomodulator will drive complications.
Keywords:. Polycystic ovary syndrome, PCOS, obesity, progesterone-induced blocking factor, PIBF, inflammation.

Anahtar kelimeler: Polikistik over sendromu, progesteron kaynaklı bloke edici faktör, pibf, inflamasyon, obezite, PKOS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an important endocrinopathy affecting 5–15% of women during their reproductive ages[1]. Although the clinical results vary depending on the age of diagnosis, the short-term findings are menstrual irregularities, infertility, obesity, and insulin resistance. In the long term, increased incidences of coronary artery disease, dyslipidemia, cerebrovascular disease and metabolic syndrome have been documented[2]. PCOS is characterized by systemic inflammation caused by endothelial dysfunction and increased oxidative stress, and the number of studies on the role of immune mechanisms in the presence of PCOS are increasing[3]. Furthermore, PCOS represents chronic inflammation status with permanently elevated levels of inflammatory markers including interleukin (IL)-6 and IL-18, tumor necrosis factor (TNF)-α, and C reactive protein (CRP)[4]. Studies reporting an increase in T-helper (Th)-1 inducer TNF-α in follicular fluid have focused on immune regulation in the pathogenesis of PCOS[5]. Besides, studies investigating the etiopathogenesis of PCOS have shown that immune regulation shifts from a balance of Th1/Th2 to Th1-dominant immunity[6].

Progesterone-induced blocking factor (PIBF) is an immunosuppressive factor synthesized by lymphocytes[7]. PIBF is a progesterone-derived protein that has immunological effects on the Th1/Th2 cascade within the immune system, maternal natural killer (NK) cells, and the asymmetric antibody system, which masks fetal antigens[8]. There are various isoforms of PIBF. The parent form measures 90 kilodaltons and is localized in the centrosome. Various alternative splicing variants of this paternal form elicit smaller intracytoplasmic molecules with immunosuppressive activity[9].

In the presence of PIBF, during normal pregnancy development, proinflammatory Th1–dependent cytokine dominance (interferon-gamma (IFN-γ), TNF-α, IL-2, IL-12, and IL-18) shifts to anti-inflammatory Th2–dependent cytokines (IL-3, IL-4, IL-6, IL-10, and IL-13)[10]. PIBF has been shown to have a role in healthy pregnancies by activating Th2 and inhibiting NK cells[11]. In addition, studies have shown that PIBF levels are low in cases of unexplained infertility and underlying mechanism is decreased Th2 ratios and activation of NK cells[12].

PIBF and PCOS are both functioning in the Th1/Th2 cascade pathway. The relationship between PCOS and PIBF level is important when considering their working mechanisms. Considering the complications that occur in PCOS, a low PIBF level can be considered as the reason. In this study, it was aimed to investigate the level of PIBF, an immune mediator, in patients with PCOS.

MATERIALS AND METHODS

The present research is a cross-sectional study conducted at Tuzla State Hospital in Istanbul/Turkey. The Marmara University Faculty of Medicine Ethics Committee approved the study protocols (approval no: 09.2020.1140). It is carried out in accordance with the Declaration of Helsinki and informed consent was obtained from the participants.

Sample

The study participants were 18–35 years old, were not obese (body mass index (BMI) < 25 kg/m^2), and all applied to our clinic with the complaints of acne, hirsutism or menstrual irregularity.

The diagnosis of PCOS was made according to Rotterdam diagnostic criteria and PCOS was diagnosed if two of the following criteria were met: 1) clinical or biochemical hyperandrogenism, 2) chronic ovulatory dysfunction or 3) polycystic ovarian morphology. The exclusion criteria were pregnancy, BMI > 25 kg/m^2, non-classical congenital adrenal hyperplasia, Cushing's disease, hyperprolactinemia, known thyroid disease, or other systemic disease.

The number of patients in our study was determined by reference to the previous study by Gong et al. [6]. The study consisted of two groups, patients diagnosed with PCOS (n = 36) and a healthy control group (n = 36).

Clinical and hormonal analysis

Demographic characteristics of the patients, such as weight, height, age, and BMI were recorded. The Ferriman-Gallwey score was used for hirsutism[13]. The Global Acne Grading System (GAGS) score was used for acne problems[14]. Ferriman-Gallwey score, GAGS, weight changes, and the number of menstrual cycles within the last year were evaluated.

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), prolactin (PRL), total testosterone, and dehydroepiandrostenedione sulfate (DHEA-S) levels were measured in the biochemistry laboratory of Tuzla State Hospital on the third day of the menstrual cycle. On the 21st day of the same menstrual cycle, fasting blood glucose, insulin, and progesterone levels were also measured. Fasting insulin (μU/mL) * fasting glucose (mg/dL) / 405 formula of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was used for the assessment of insulin resistance [15]. To analyze PIBF levels, 5 mL of venous blood was drawn and placed into serum-separating biochemistry tubes, after which they were centrifuged at 1000 rpm at 4°C for 10 min. The supernatant from the samples was transferred into clean 1.0 mL Eppendorf tubes and stored at -20°C until testing with enzyme-linked immunosorbent assay (ELISA). PIBF levels in the serum were measured using the Human PIBF ELISA Kit (BT-LAB E3714Hu).

Statistical analysis

Statistical analyzes were performed using SPSS 22.0 software. The conformity of the variables to the normal distribution was examined by visual (histogram) and analytical methods (Kolmogorov Smirnov/Shapiro Wilk tests). In descriptive analyses, mean and standard deviation values were given for normally distributed variables, and median and 25-75 percentile values were given for non-normally distributed variables. Student's t test was used to evaluate the relationship for age, BMI, number of menstrual cycles in the last year, GAGS, weight gain in the last year, Ferriman-Gallwey scoring variables in the case and control groups, and the Mann Whitney U test was used to evaluate the differences in biochemical parameters, progesterone and PIBF levels. A p-value of less than 0.05 was considered to show statistically significant results.

RESULTS

The present study included 72 participants, 36 of whom were PCOS patients and 36 of whom were healthy. The participant's age, weight, height, BMI, Ferriman-Gallwey score, GAGS, weight gain, and the number of menstrual cycles within the last 1 year were similar between the groups. (p= 0.819, p= 0.412, p= 0.857, p= 0.541, p= 0.115, p= 0.258, p= 0.758, p= 0.110, respectively) (Table 1). Table 2 shows the distribution of the biochemical parameters of the two groups. Serum FSH (p= 0.711), E2 (p= 0.845), TSH (p= 0.882), PRL (p= 0.330), DHEA-S (p= 0.536), total testosterone (p= 0.283), fasting blood glucose (P= 0.728, fasting insulin (p= 0.082), HOMA-IR (p= 0.076), and hemoglobin A1c (HBa1c) (p= 0.078) values were similar between the two groups. Although serum LH (p= 0.004) and LH/FSH values ($p < 0.001$) were higher in the PCOS group than in the control group, serum progesterone (p= 0.003) levels were significantly lower in the PCOS group. The PIBF value was 4.64 ± 7.08 in the PCOS group and 15.40 ± 2.99 in the control group. This was also statistically significant (p= 0.001).

Table 1. Comparison of demographic and clinical characteristics between groups.

Characteristic	Polycystic Ovary Syndrome Group (n: 36)	Control Group (n: 36)	P-value
Age (years)	23.81 ± 4.97	23.56 ± 4.23	0.819
Body mass index (kg/m^2)	23.56 ± 0.91	23.02 ± 0.52	0.541
Number of menstrual cycles in the last year	7.51 ± 1.22	9.81 ± 0.95	0.110
Global Acne Grading System	18,02 ± 4.85	15.15 ± 5.02	0.258
Weight gain in the last year	2.11 ± 0.57	1.98 ± 0.78	0.758
Ferriman–Gallwey score	7.08 ± 0.42	5.45 ± 1.02	0.115

Table 2. Comparison of biochemical parameters, progesterone, and progesterone-induced blocking factor (PIBF) levels between groups.

Biological Parameters	Polycystic Ovary Syndrome Group (n: 36)	Control Group (n: 36)	p-value*
FSH (IU/L)	6.00 (5.11-6.82)	6.44 (5.10-7.2)	0.711
LH (IU/L)	8.53 (5.99-10.34)	5.81 (3.95-7.97)	0.004
LH/FSH	1.50 (0.95-1.80)	0.92 (0.77-1.13)	<0.001
Estradiol (pg/mL)	35.15 (29.48-49.15)	34.95 (23.61-47.33)	0.844
TSH (mIU/L)	1.95 (1.48-3.10)	2.00 (1.49-2.88)	0.822
PRL (µg/L)	21.08 (15.62-28-97)	21.95 (13.88-36.64)	0.330
DHEA-S (µg/dL)	237.90 (196-347.75)	221.50 (188.10-301)	0.536
Total testosterone (ng/L)	0.43 (0.31-0.53)	0.34 (0.27-0.50)	0.283
Fasting glucose (mg/dl)	94.1 (89.25-97.75)	92.00 (88.00-96.82)	0.728
Fasting insülin (mIU/L)	11.35 (9.31-13.17)	9.35 (7.04-11.19)	0.082
HOMA-IR	2.53 (2.18-3.02)	2.16 (1.55-2.59)	0.085
HBa1c	5.30 (5.12-5.50)	5.25 (5.02-5.40)	0.078
Progesterone (µg/L)	0.58 (0.32-2.05)	3.24 (0.47-9.25)	0.003
PIBF (ng/dL)	199.09 (166.09-398.06)	619.83 (267.26-2354.32)	<0.001

FSH: Follicle-stimulating hormone LH: luteinizing hormone TSH: Thyroid-stimulating hormone PRL: Prolactin DHEA-S: dehydroepiandrosterone sulfate Hba1c: Hemoglobin A1c PIBF: Progesterone-induced blocking factor Values are presented median (inter-quartile range). * Comparison between groups was made by the Mann–Whitney U test or Student T-test

DISCUSSION

PCOS is among the most common endocrine diseases that affect women during their reproductive age. Its immune regulation causes a shift from a balance of Th1/Th2 to Th1-dominant immunity. The present study aimed to evaluate maternal serum progesterone and PIBF levels that regulate the Th1/Th2 balance in non-obese women with PCOS. The key findings were as follows: (1) serum progesterone levels were significantly lower in the PCOS group than in the control group; (2) serum PIBF levels were significantly lower in the PCOS group than in the control group; and (3) fasting blood glucose, fasting insulin, HOMA-IR, BMI, total testosterone, DHEA-S, Ferriman–Gallwey score, and weight gain in the last year were similar between the two groups.

The risk factors for PCOS are with a sedentary lifestyle and western eating habits, which result in fat accumulation and further effects on the immune system[16]. Immunologic outcomes are described as a mild but chronic proinflammatory condition that affects not only adipose tissue but also other target organs, such as the ovaries, in obese patients[17]. Studies have shown that a higher BMI in PCOS patients is associated with hypertriglyceridemia, which is related to obesity-induced adipokine alteration, including TNF-α, IL-6, and adiponectin[18,19].

Gong et al.[6], in their study investigating obesity and immune balance, have shown that the Th1/Th2 ratio increases in PCOS patients, proinflammatory cytokines increase more in the presence of obesity, and the Th1/Th2 ratio changes accordingly. In addition, studies on ovaries in PCOS patients have reported that low-grade inflammation is observed with PCOS, with the detection of polymorphonuclear leukocytes in ovarian tissue and follicle fluids, and immune regulation is one of the basic elements of PCOS[20-22]. Considering the effect of obesity on proinflammatory cytokines, especially obese and overweight patients were excluded from the present study. Hence, we found that fasting blood glucose, insulin, HOMA-IR, BMI, total testosterone, DHEA-S, Ferriman–Gallwey score, and weight gain over the last year were similar between the groups.

More importantly, we found that serum progesterone and PIBF levels were significantly decreased in the PCOS group compared to that in the control group. It is well documented that PIBF is a progesterone-derived protein with immunological effects that shifts Th1–dependent cytokine dominance (TNF-α, IFN-γ, IL-2, IL-12, and IL-18) to anti-inflammatory Th2–dependent cytokines (IL-3, IL-4, IL-6, IL-10, and IL-13)[8,10]. Considering that obesity and

hyperandrogenemia had no confounding effect on inflammatory regulation in the present study, it is possible to say that the predominance of Th1 immunity in these patients is because of decreased progesterone and PIBF levels. To the best of our knowledge, the present study is the first to evaluate PIBF levels in non-obese women with PCOS.

The findings of the present study have clinical importance that can be applicable in routine practice. First, infertility and recurrent miscarriages are among the clinical complications of PCOS, andmay be the result of decreased immunotolerance due to decreased PIBF levels in PCOS patients. Jakubowicz et al.[23] have reported that miscarriages early in pregnancy were 40% higher in women with PCOS. In another study by Sagle et al., PCOS was detected in 82% of women presenting with recurrent miscarriages[24]. In their study examining PIBF and Th1/Th2 cytokines, Hudic et al.[25] have found that PIBF levels in urine and serum are significantly lower in PCOS patients compared to that in the control group, and they argued that PIBF is crucial for a healthy continuation of pregnancy. In their study on PIBF, which is thought to be a part of the immune response necessary for the recognition and continuation of pregnancy, and in which its role in unexplained infertility was demonstrated, Eraslan Sahin et al.[12], have found that serum PIBF levels are lower in the infertility group. Complications that occur after a decrease in PIBF level in PCOS patients, as demonstrated in the present study, were also demonstrated in previous studies. Second, the clinical importance of progesterone supplementation in in-vitro fertilization applications in infertility treatments is well known[26]. Considering that PIBF is a progesterone-mediated immune mediator, progesterone supplementation may increase PIBF levels and improves perinatal outcomes when women with PCOS become pregnant. Last, in the presence of immune imbalance, the pregnancy is complicated by preeclampsia, intrauterine growth restriction, and small-for-gestational-age fetuses characterized by insufficient placentation. Studies conducted by Hu et al.[27] have shown an increased risk ofhypertension during pregnancy and preeclampsia in women with PCOS. In their study that examined PIBF levels and pregnancy outcomes, Polgar et al.[28] have shown that serum and urinary PIBF levels are lower in women with preeclampsia, the threat of miscarriage, and the threat of preterm birth, dysmaturity, and polyhydramnios complications. Since the most important immunological determinant of placentation is the level of PIBF, it appears to be related to these outcomes in these patients; however, more advanced prospective studies are necessary to determine the clinical significance of these statements and recommendations.

In the present study, only non-obese patients were included; therefore, the inflammatory condition caused by obesity was eliminated. The absence of obesity and insulin resistance were similar between the two groups and no difference was found in the androgen and E2 levels between the groups, which are believed to play a role in the inflammatory process. Because PIBF and progesterone levels were significantly different between the groups, we observed that PIBF was the underlying mechanism of PCOS complications.

Our study has some limitations such as the fact that it was conducted in a single center, limited number of patients were reached, and inflammation parameters (IL levels; Th1, Th2 expression, TNF-α level, IFN-γ level) could not be evaluated.

Our results indicated that progesterone and PIBF levels decreased in non-obese PCOS patients. We concluded that even in the absence of obesity, which is the origin of inflammation and exacerbates the condition in PCOS patients, low PIBF as the underlying immunomodulator will form the basis for further complications. Additional prospective studies are needed to support the clinical significance of our findings.

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