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Evaluation of Endometrial Bcl-2 Expression and Ki-67 Proliferative Index in Infertile Patients with and without Polycystic Ovary Syndrome

Dilay Karademir[1a,*], Akın Usta[2a], Eren Altun[3a]

[1]*Sivas Cumhuriyet University, Medical Faculty, Department of Obstetric and Gynecology, Sivas, TURKEY*

[2]*Balıkesir University, Medical Faculty, Department of Obstetric and Gynecology, Balıkesir, TURKEY*

[3] *Balıkesir University, Medical Faculty, Department of Pathology, Balıkesir, TURKEY*

**Corresponding author*

Review Article

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ABSTRACT

Background: In women of reproductive age, polycystic ovary syndrome (PCOS) is the most prevalent cause of infertility. The purpose of this study is to compare the Bcl-2 and Ki-67 values between infertile patients with and without PCOS.

Methods: The study included 27 infertile patients diagnosed with PCOS and 28 infertile patients who did not meet the criteria for PCOS. Smoking, pelvic infection symptoms, endometrial polyps and submucosal myomas during a transvaginal ultrasound, pituitary insufficiency, hyperprolactinemia, congenital adrenal hyperplasia, having had adnexal surgery and having a male factor that will result in infertility are all considered exclusion criteria. All patients' data were collected, including age, the length of their infertility, body mass index (BMI), waist-to-hip ratio (WHR), hirsutism score, blood pressure, total testosterone, triglyceride, total cholesterol, LDL, and HDL values, as well as Homa-IR and Hs-CRP readings. The pathology specialist in the examples evaluated Bcl-2 and Ki-67 levels.

Results: We found that BMI, WHR, total testosterone level, blood pressure, total cholesterol, HOMA-IR, and hs-CRP values were significantly higher in infertile cases with PCOS. We also found that the Ki-67 and Bcl-2 values were higher in endometrial cells in sterile PCOS cases than in the control group.

Conclusions: Ki-67 and Bcl-2 levels rise in PCOS patients, preventing apoptosis, limiting the formation of a suitable endometrial environment, and preventing embryo implantation. PCOS patients frequently experience infertility and recurrent pregnancy losses. The cause of this problem may be the increased activity of estrogen. The primary treatment for PCOS will depend on further investigation into the variables that affect GnRH release, and the care plan should be built around this goal.

Keywords: Apoptosis, Bcl-2, Infertility, Ki-67, Polycystic Ovary Syndrome

Polikistik Over Sendromu Olan ve Olmayan İnfertil Olgularda Endometrial Bcl-2 Ekspresyonu ve Ki-67 Proliferatif İndeksinin Değerlendirilmesi

Süreç

Geliş: 12/02/2023
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ÖZ

Amaç: Polikistik over sendromu üreme çağındaki kadınlarda görülen en sık infertilite nedenidir. Bu çalışmanın amacı; polikistik over sendromu olan ve olmayan infertil hastalardaki Bcl-2ve Ki-67 değerleri arasındaki farkı biyokimyasal parametreler eşliğinde incelemektir.

Yöntem: Çalışmaya polikistik over sendromu teşhisi konulan 27 infertil hasta ve polikistik over sendromu kriterlerine uymayan 28 infertil hasta dahil edildi. Çalışmadan dışlanma kriterleri; kişinin sigara kullanması, pelvik enfeksiyon bulgusu, transvajinal ultrasonografide endometrial polip, submüköz myom vb. saptanması, hipofizer yetmezlik, hiperprolaktinemi, konjenital adrenal hiperplazisi olmak, adneksiyal cerrahi geçirmiş olmak ve infertiliteye sebep olacak erkek faktörü bulunmaktır. Tüm bireylerin yaş, infertilite süresi, vücut- kitle indeksi, bel-kalça oranı, hirsutizm skoru, kan basınçları, total testesteron, trigliserit, total kolesterol, LDL, HDL değerleri, HOMA-IR ve hsCRP değerleri not edildi. Patoloji uzmanı tarafından Bcl-2ve Ki-67 düzeyleri değerlendirildi.

Bulgular: PKOS'lu infertil olgularda vücut- kitle indeksi, bel-kalça oranı, total testosteron seviyesini, kan basıncını, total kolesterol değerini, HOMA-IR ve hs-CRP değerlerini belirgin yüksek bulduk. Ayrıca Ki-67 ve Bcl-2 değerinin PKOS'lu infertil olgularda endometriyal hücrelerde kontrol grubuna oranla daha yüksek olduğunu bulduk. **Sonuç**: Ki-67 ve Bcl2 değerleri, PKOS'lu olgularda artarak, apopitozisin gerçekleşmesini engeller ve uygun endometriyal ortam oluşmasını engelleyerek de embriyonun implantasyon sürecini kısıtlar. Bu durum PKOS'lu olgularda infertilite ve tekrarlayan gebelik kayıplarına neden olmaktadır. Bu soruna artmış östrojen aktivitesi sebep olmaktadır. PKOS'un ana tedavisinde özellikle GnRH salınımını düzenleyici ajanlar üzerine çalışmalar yapılmasına ihtiyaç duyulduğu ve tedavinin bu hedef üzerine şekillenmesi gerektiği kanaatindeyiz.

Anahtar sözcükler: Apopitozis, BCL-2, İnfertilite, Kİ-67, Polikistik Over Sendromu

[a] *drdilaykarademir@gmail.com* *https://orcid.org/0000-0002-9813-4255*
[b] *drakinusta@gmail.com* *https://orcid.org/0000-0001-8973-4374*
[c] *erenaltun@hotmail.com* *https://orcid.org/0000-0001-9110-8364*

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in reproductive women[1]. Although its prevalence varies according to societies and diagnostic criteria, it is between approximately 8 % to 13 % [2]. Clinical features of PCOS on ultrasound include polycystic ovarian appearance, oligo/anovulation, hormonal and clinical hyperandrogenemia, and infertility [3]. It is a complex disease whose etiopathogenesis is accused of factors such as genetic sensitivity, epigenetic mechanisms, insulin resistance, environmental effects, steroid metabolism changes, and lifestyle [4]. Etiology is still not fully illuminated. Due to all these etiological reasons, the frequency and amplitude of luteinizing hormone (LH) release increase due to insensitivity to gonadotropin-releasing hormone (GnRH) at the pituitary level. The rise in LH is more significant than the increase in follicle-stimulating hormone (FSH). This increase in LH stimulates androgen synthesis in theca cells. Impaired FSH synthesis and release leads to insufficient stimulation of granulosa cells, resulting in decreased aromatase activity. This condition causes an increase in circulating androgen levels [5]. Hirsutism and obesity resulting from hyperinsulinemia and hyperandrogenemia are among the leading physiological causes of PCOS [6]. PCOS may be associated with severe health issues such as diabetes, coronary heart disease, and cancer. Furthermore, endometrial hyperplasia and endometrial cancer are more likely to develop when estrogen is not balanced by progesterone[7,8]. PCOS, a metabolic syndrome, is an essential reproductive disorder and is the most common cause of infertility due to anovulation in reproductive women [9].

It has been shown that PCOS increases preeclampsia in pregnancy by 3 to 4 times and causes miscarriages, gestational diabetes mellitus (DM), and premature births. This situation suggests that PCOS disrupts the placentation process [10,11]. Fauser et al. have noted changes in many biochemical markers associated with chronic inflammation, endothelial dysfunction, hyperandrogenism, dyslipidemia, obesity, and insulin resistance in patients with PCOS [7]. Studies show that hyperandrogenism and insulin elevation can cause changes in endometrial functions by affecting the expression of growth factor receptors and steroid receptors [10,12].

Due to these changes caused by PCOS, homeostasis is disrupted throughout the body. Programmed cell death is an essential regulatory mechanism in achieving homeostasis; apoptosis is also affected. Achieving a normal endometrial cycle depends on the balance between apoptosis and mitosis [13]. The increase or decrease of apoptosis can cause many diseases. Patients diagnosed with PCOS increased the Bcl-2/Bax ratio due to the release of estrogen not being met with progesterone in the endometrium [8].

This study aimed to compare histopathological Bcl-2 and Ki 67 expression rates in endometrial tissue samples in infertile patients with and without PCOS and to evaluate them biochemically in light of hormonal parameters.

Materials and Methods

The Balıkesir University Faculty of Medicine Clinical Research Ethics Committee approved this prospective study. Sixty patients aged 18-45 who applied to Balıkesir University Obstetrics and Gynecology Clinic with infertility complaints were selected for the study. A detailed informed consent form was obtained from all participants. Each patient was given detailed information about the study to be performed, and their verbal and written informed consents were obtained. 2 patients were removed from the control group, and three patients were removed from the study group because they did not meet the research criteria. Patients were divided into two groups 27 infertile patients diagnosed with PCOS and 28 infertile patients with normal endometrial findings who did not meet the requirements of PCOS. The study group of infertile patients diagnosed with PCOS based on 2003 Rotterdam ESHRE/ASRM consensus criteria [14]. Patients with normal endometrial findings diagnosed with unexplained infertility constituted the control group criteria for excluding volunteers from research. Cases with endometrial polyps, submucosal myoma, pathology detection in transvaginal ultrasonography or endometrial sampling by the patient, endocrine diseases such as pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia, ovarian or adnexal surgery and male factor that will cause infertility were accepted as cases with male factors that would cause infertility and the instances that voluntarily left the study were excluded from the study. In these cases, age, body mass index (BMI), waist-hip ratio (WHR), hirsutism score, blood pressure, and duration of infertility were recorded.

Biochemical evaluation

5cc venous blood was taken from all patients for basal hormonal evaluation. After centrifugation (850g, 10 min), serum samples were obtained, emptied into Eppendorf tubes (Eppendorf, Hamburg, Germany), and stored in a deep freezer at 80 degrees Celsius until biochemistry analysis was performed. Serum samples were analyzed by a complete quantitative method. Follicular Stimulant hormone (FSH, mIU/mL), Luteinizing hormone (LH, mIU/mL), Estradiol (E2, pg/mL), Total testosterone (ng/dl), Triglyceride (mg/dl), Total cholesterol (mg/dl), LDL (mg/dl), HDL (mg/dl) were analyzed by complete quantitative method (Cobas Integra 800; Roche Diagnostics GmbH; Mannheim, Germany), hs-CRP was evaluated using the chemiluminescent immunoassay method (ADVIA Centaur XP, Siemens Healthcare Diagnostics, NY, USA).

Insulin values were studied with hormone auto-analyzer devices (Beckman Coulter; Unicel DXI 600; Access Immunoassay System). HOMA-IR (mg/dl) (fasting insulin x fasting glucose)/ (constant) was calculated with the formula. Since the fasting glucose value was calculated as mg/dl, the constant was taken as 450, and the limit value was accepted as 2.4.

Hysteroscopic endometrial biopsy sampling

Hysteroscopic endometrial biopsy sampling with sedation was taken from all the patients who participated in the study. Sample endometrial tissues were first followed by light microscopy. Then an immunohistochemistry study was performed to mark them with hematoxylin-eosin (H-E) stain and Ki-67 and Bcl-2.

Light microscopy and Immunohistochemical evaluation

1. Endometrial tissue samples were fixed 24 hours daily in 10% formaldehyde.
2. Sections were passed through increasing ethanol series.
    - 90% ethanol.....1 day
    - 96% ethanol.....1 day
    - Pure ethanol ..... 1 day
    - Toluol .....1 hour
3. The tissues were kept in liquid paraffin at 35°C temperature twice for 1 hour to prepare for cross-section. The tissues were then embedded in paraffin inside square-shaped iron blocks. The paraffin blocks with tissue were removed from the iron blocks after cooling. 4-micron endometrial tissue sections taken from paraffin blocks using microtome were taken separately for each staining on 1/10 poly-L-lysine treated slides. The sections were kept in a 56°C oven for the night to remove the paraffin. They were secondarily put through xylene and decreasing ethanol series to remove the paraffines. Toluol 2x30 minutes, Pure ethanol 10 minutes, 96% ethanol 10 minutes, 90% ethanol 10 minutes, 70% ethanol 10 minutes, distilled water 2x5 minutes, sections were kept in the dark for 20 minutes in 1% H2O2 prepared with methanol to prevent endogenous peroxidase activities. The sections were then renewed in phosphate buffer containing 0.1 % triton-X-100 and kept times for 5 minutes. The sections were transferred to a plastic shawl containing ten10 mM citrate buffer (pH:6) to ensure antigen reversal, and the citrate buffer was renewed each time in the microwave oven and kept three times for 2 minutes. The sections removed from the microwave oven were allowed to cool for 20 minutes at room temperature. After threats, the protein block solution containing 0.1% triton-X-100 was renewed in PBS and kept three times for 5 minutes. The sections were placed in the immunohistochemistry and were drawn with a hydrophobic pencil. To prevent non-specific attachment to this area, chitin that closes the epitopes was administered in PBS for 5 minutes. Commercially available primary Ki-67 and Bcl-2 antibodies (1:100 dilution) were added to the sections without removing PBS and refrigerated at four °C for 12 hours. The sections were then renewed each time on PBS and three times for 5 minutes. The sections were then administered a biotin secondary antibody for 1 hour. The sections were then restored on PBS and times for 5 minutes each time. After the sections, streptavidin peroxidase was administered for 30 minutes. The cells were then renewed on PBS and kept twice for 5 minutes. Diaminobenzidine (DAB) chromogen amino ethyl cortisol prepared for sections was administered under a microscope in the dark. In the last stage, Mayer's hematoxylin was applied to threads for 5 minutes for posterior area painting. The sections were soaked in tap water for 15 minutes, then taken into distilled water. After removing water from the sections, the DAB-compatible mounting medium was dripped onto them and covered with lamellae. The sections were imaged using a digital camera and counted using a light microscope. In evaluating Bcl-2 expression, the intensity of positive staining in glandular cells was assessed through the assessment Ki-67, and areas with the highest proliferative activity were selected, which did not include hemorrhage and necrosis from the materials. Ki67 and Bcl-2 expression were examined and scored semi-quantitatively according to staining intensity and size; Ratings were recorded as non-staining (0), (1+) for weak positive staining, and (2+) for intense positive staining.

Statistics

SPSS 22.0 for the Windows program was used for statistical analysis. In assessing the data obtained from the survey, Chi-square, Man Whitney U, and independent T-test were used to determine the difference between the two means in separate groups. The Spearman Rank test evaluated the correlation between the groups. $P < 0.05$ level was significantly assessed.

Results

Patients were divided into two groups (PCOS and control group); descriptive data, including age, BMI, WHR, hirsutism score, blood pressure, and FSH, LH, E2 total testosterone, triglycerides, total cholesterol, LDL,

HDL, HOMA-IR, and hs-CRP values are compared in Table 1.

Table 1. Comparison of PCOS and Control group descriptive parameters

	PCOS group (n:27)	Control group (n:28)	*P*
Age (min-max)	25 (18-34)	26 (21-37)	0.1579
BMI (kg/m^2)	25.3 (19.9-37.8)	21.9 (17.5-27.4)	**< 0.001**
WHR	0.87 (0.70-0.95)	0.74 (0.65-0.97)	**< 0.001**
Hirsutism score	11 (2-14)	1,6 (1-7)	**< 0.001**
Systolic blood pressure (mmHg)	130 (90-140)	100 (90-120)	**< 0.001**
Diastolic blood pressure (mmHg)	80 (60-90)	60 (50-80)	**< 0.001**
FSH (mIU/mL)	6.7 (3.3-10.3)	6.2 (3.2-10.5)	0.9664
LH (mIU/mL)	6.4 (2.2-20.8)	4.8 (2.8-10.0)	**< 0.001**
E2 (pg/mL)	41 (7-120)	29 (14-92)	**< 0.001**
Total Testosteron (ng/dL)	0,52 (0.24-3.26)	0.11(0.07-0.25)	**< 0.001**
Triglycerides (mg/dL)	124 (45-276)	67(44-109)	**< 0.001**
Total cholesterol (mg/dL)	179 (109-257)	173 (107-226)	0.3813
LDL (mg/dL)	99 (64-158)	96 (61-138)	0.2965
HDL (mg/dL)	50 (37-77)	63.5 (36-82)	**0.001**
HOMA-IR	2,62 (0.95-17.02)	1.44 (0.46-5.36)	**0.001**
hs-CRP	3.1(0.3-39.8)	1.1 (0.2-9.8)	**< 0.001**

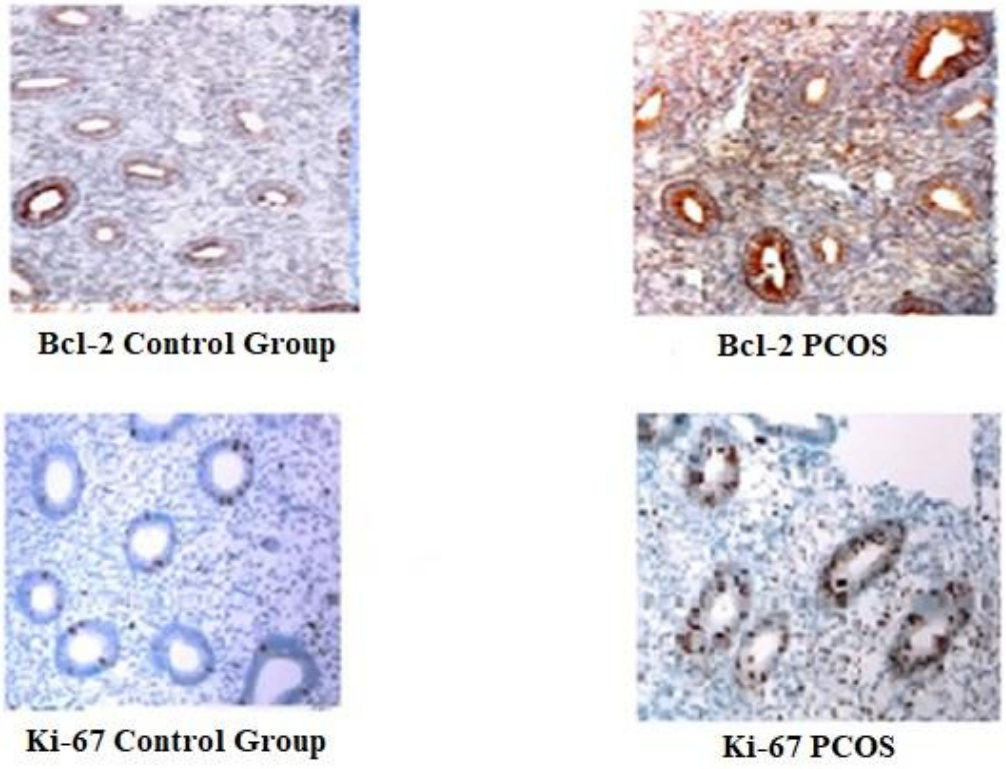
Mann-Whitney test, x^2 test, mean±SEM or median (min-max)

Twenty PCOS patients were during the proliferative phase, while seven were during the secretory phase. There were also nineteen cases in the proliferative phase and nine in the secretory phase in the control group. No statistically significant difference also existed between the groups (p=0.2956).

Light microscopic images of Bcl-2 gene expression and Ki-67 proliferative index in endometrial tissue sampling of patients in both groups are given in Figure 1.

Even while the duration of infertility was 3 (1-6) years in patients with PCOS, it was calculated as 2 (1-8) years in the control group. In terms of the duration of infertility, there was no significant difference between the groups. (p=0.2738).

Figure 1: Bcl-2 gene expression and Ki-67 proliferative index in endometrial biopsy



Light microscopy imaging in PCOS and Control Group

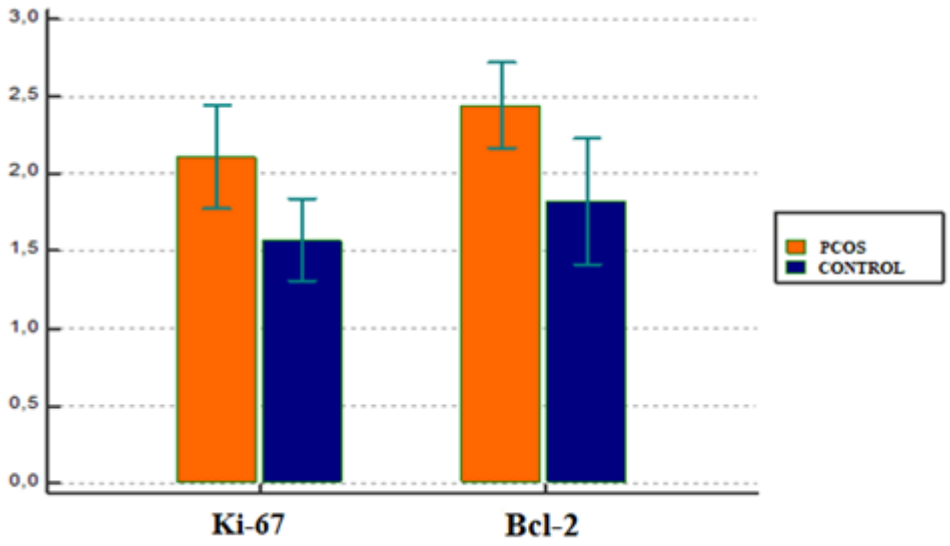
The mean Ki-67 value was determined as 2.1±0.16 in PCOS patients, compared to 1.57±0.13 in the control group. When both groups reached the Ki-67 proliferative index value, the Ki-67 proliferative index value was statistically significantly higher in the PCOS group (p=0.0122). The mean Bcl-2 value in patients with PCOS was 2.46±0.12. In the control group, the mean Bcl-2 value was calculated as 1.78±0.20. Bcl-2 antiapoptotic gene expression was statistically significantly higher in the PCOS group compared to the control group (p=0.0059) (Table 2; Figure 2).

Table 2: Bcl2 and Ki-67 expression values in PCOS and Control groups

	PCOS	Control group	*P*
Kİ-67 expression	2.11±0.16	1.57±0.13	**0.0122**
Bcl-2 expression	2.46±0.12	1.78±0.20	**0.0059**

Independent t-test

Figure 2: Ki-67 and Bcl-2 gene expression in the PCOS group and Control group



Spearman's correlation analysis of PCOS and Control group descriptive parameters shows a positive and significant association between BMI, WHR, Total testosterone, HOMA-IR, and hs-CRP with Bcl-2 antiapoptotic gene expression and Ki-67 proliferative index; the important negative relationship was observed with HDL. The data for the analysis are shown in Table 3.

Table 3. Spearman correlation of descriptive parameters with Ki 67 and Bcl2 expression

	Ki-67		Bcl-2	
	$r_{spearman}$	*p*	$r_{spearman}$	*p*
Age (years)	0.260	0.0550	0.159	0.2463
BMI (kg/m²)	0.5689***	**<0.001**	0.349**	**0.0090**
WHR	0.366**	**0.006**	0.359**	**0.0071**
Hirsutism score	0.226	0.0974	0.263	0.0528
Systolic blood pressure (mmHg)	0.136	0.3212	0.269*	**0.0467**
Diastolic blood pressure (mmHg)	0.229	0.0929	0.199	0.1456
Total testosterone (ng/dL)	0.307*	**0.0225**	0.340*	**0.0111**
Triglycerides (mg/dL)	0.254	0.0611	0.4687***	**<0.001**
Total cholesterol (mg/dL)	0.0523	0.7048	0.0800	0.5615
LDL (mg/dL)	0.0764	0.5794	0.0915	0.5065
HDL (mg/dL)	- 0.252	0.0635	- 0.389**	**0.0033**
HOMA-IR	0.415**	**0.0016**	0.474***	**<0.001**
hs-CRP	0.269*	**0.0470**	0.630***	**<0.001**

* p<0.05. ** p<0.01. *** p<0.001

Discussion

In our study, we examined the effects of Bcl-2 antiapoptotic gene expression and Ki 67 antigen, a marker of mitotic activity, on prognosis in the light of hormonal parameters and compared Bcl-2 antiapoptotic gene expression in endometrial tissue samples taken from infertile patients with and without PCOS.

In obese women with PCOS, insulin resistance changes the expression of endometrial estrogen, androgen, and progesterone receptors [9]. Palomba et al. found endometrial resistance to progesterone in PCOS patients and abnormal gene expression associated with increased estrogen activity due to increased estrogen receptor expression [15]. So it has been reported that hyperinsulinemia can impact the endometrium, resulting in inadequate epithelial differentiation during the first few weeks of pregnancy [16]. Yet it has been shown that obesity, insulin resistance, or diabetes increases the risk of endometrial thickness, irregular uterine bleeding, and even endometrial cancer in PCOS patients who are not pregnant [17]. Android-type obesity is a known significant risk factor for developing Type 2 DM and cardiovascular disease (CVD) in the long term. Solomon et al. showed that the transition from impaired glucose tolerance to type 2 DM increased 2–5 times in obese PCOS patients in the USA and Australia [18]. Also, in a research of 1741 cases evaluating the prevalence of obesity in PCOS, 38% of the patients had the disease [19]. According to the literature, in our study, patients with PCOS had significantly higher BMI levels than the control group and HOMA-IR and E2 values as indicators of insulin resistance. Menstrual irregularity and BMI were found to be strongly correlated. Although these features are the main factor in the aggravation of the symptoms of PCOS patients, they indicate that PCOS is both an endocrinological and metabolic disease.

Insulin resistance and hyperinsulinemia in PCOS cause a decrease in SHBG (sex hormone binding globulin) levels and increase androgen synthesis and free testosterone levels in the ovary. In a study conducted on 264 women with PCOS in India, patients were divided into two groups: those with glucose intolerance and those without, using the OGTT 2nd hour value, and HOMA-IR and total testosterone values were found to be higher in the group with abnormal glucose tolerance [20]. In this study, total testosterone levels in infertile patients with PCOS were significantly higher than in the control group, consistent with the literature. The increased thecal thickness in PCOS leads to an increase in testosterone activity and expression, as well as the inhibitory effect of high Anti Müllerian Hormone release (AMH) on aromatase activity caused by FSH, which leads to a decrease in testosterone conversion to estrogen and an increase in testosterone levels [21]. In conclusion, this situation contributes to the development of infertility with the mechanisms that occur due to anovulation and insulin resistance. However, further studies at the molecular level are needed.

PCOS patients are at risk for many chronic diseases, especially CVD and DM. C-reactive protein (CRP), an acute phase reactant, is a highly sensitive marker of chronic low-grade inflammation and is closely associated with CVD and DM [22]. In the literature, many studies have shown increased CRP values in PCOS and suggested that PCOS is an inflammatory disease [23,24]. Karoli et al. stated that there was no increase in CRP values in women with PCOS compared to the control group [25]. Again, Ganie et al. did not find a significant difference in CRP values compared to the control group in their study with 160 PCOS patients [22]. Studies show that ovarian androgen levels increase in infertile patients with PCOS in correlation with CRP levels, which are markers of inflammation [26]. Increased inflammatory activity in patients with PCOS may affect folliculogenesis and lead to infertility by anovulation [27]. In our study, supporting this theory, the hs-CRP value was statistically significantly higher in the infertile group with PCOS than in the control group. Nevertheless, considering the small sample size in our study, there is a need for studies with a higher number of patients in this regard.

It is known that the endometrial cycle occurs with the harmonious functioning of the mechanisms of apoptosis and antiapoptotic activity. Many studies have also shown that the action of estrogen and progesterone 4,10 hormonally regulates these mechanisms. Estrogen and progesterone regulate the uterine epithelium's proliferation, differentiation, and death. Nawaz et al. stated that a significant increase in the apoptotic index of the endometrium was observed after oophorectomy in rabbits, which could be prevented by exogenous estrogen replacement therapy [28]. Maliqueo et al. stated that in their study of women with PCOS, the synthesis of antiapoptotic Bcl-2 was significantly higher in the endometrium with PCOS due to the effect of estrogen not being met with progesterone [4]. Vaskivuo et al. stated that Bcl2 gene expression in human endometrium with regular menstrual cycle findings increases in the proliferative period due to cyclic hormonal changes and decreases with the onset of menstruation in the secretory period. The Ki-67 protein is a marker monitored in the cell nucleus during the proliferative phase of the cellular cycle. They reported that in their study, an increase in the Ki-67 index was observed due to the effect of increased estrogen in the proliferative phase of the cycle [29]. Our study evaluated cases in the proliferative phase of the menstrual cycle in infertile instances with and without PCOS. Both groups were compared in terms of the Bcl-2 and Ki-67 proliferative index. Accordingly, to the literature, increased Bcl-2 gene expression and increased Ki-67 index was detected in cases with PCOS compared to the control group.

Implantation pathologies are also efficient in infertility and early-week losses because the mechanism of apoptosis cannot be achieved because of elevated Bcl-2 expression in infertile patients with PCOS. Additionally, it should be emphasized that PCOS patients have a significant long-term risk of developing endometrial cancer due to all this.

In the current study, unlike the studies on PCOS in the literature, infertile patients with and without PCOS in the same age range were evaluated. We found that BMI was significantly higher in infertile patients with PCOS compared to the control group. Total testosterone ratio and HOMA-IR levels were elevated in infertile patients with PCOS. Increased inflammatory activity in patients with PCOS can affect folliculogenesis, cause anovulation, and disrupt the endometrial environment—raising dating the implantation of the pregnancy product and leading to infertility. The effect of increased androgens and disorders in homeostasis caused by insulin resistance contributes to the development of infertility—improved insulin resistance results in impaired glucose tolerance and obesity in patients with PCOS. In addition, this situation increases the risk of CVD in the long term for patients with PCOS. Further studies at the molecular level are needed to prove the mechanisms caused by insulin resistance, which is one of the main problems of PCOS. We found that the acute phase reactant hs-CRP was high in infertile patients with PCOS.

Conclusion

We found that; Ki-67 proliferative index and Bcl-2 antiapoptotic gene expression were significantly higher in the proliferative phase in infertile patients with PCOS. Clinically, amenorrhea and oligomenorrhea are seen in PCOS patients because apoptosis cannot take effect. In addition, this reduces endometrial receptivity and does not allow the normal implantation process of the embryo. This situation causes recurrent pregnancy losses in patients with PCOS. This picture is due to increased estrogen activity. Additionally, we think that to effectively treat PCOS, novel research on GnRH modulatory therapeutic agents, which are crucial, notably in the release of estrogen, is needed.

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