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INFLAMMATORY STATUS IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME IN GAZA STRIP

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Polycystic ovary syndrome (PCOS) is a hormonal disorder that is common among women of childbearing age. The study aimed to evaluate the inflammatory status among women with PCOS in Gaza Strip by measuring C-reactive protein (CRP) concentration and white blood cell (WBC) count in women participating in the study. 180 women were selected from gynecologic private clinics in Gaza Strip were divided into three groups: healthy control group (n= 60); newly diagnosed PCOS group (n= 60); treated PCOS group (n= 60). Our results showed significant differences in the mean concentrations of FPG, FPI, FSH, and LH in the newly diagnosed PCOS patients compared with the healthy subjects as well as the treated PCOS patients. We also observed a significant increase in CRP concentrations and WBC count in the newly diagnosed PCOS patients compared with the healthy controls (p-value< 0.001, < 0.01 respectively) as well as the treated PCOS patients (p-value< 0.01, < 0.05 respectively). In conclusion, these findings suggest that PCOS can increase insulin resistance among PCOS women which may play a role in the inflammation process among PCOS women.

Keywords: *Polycystic ovary syndrome; Insulin resistance; Inflammatory mediators*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a hormonal disorder of undefined cause which is common among women of childbearing age[1&2]. According to the World Health Organization (WHO), PCOS affects approximately 5 - 10% of premenopausal women worldwide. Women with PCOS show different clinical implications such as ovulatory dysfunction, hirsutism, type 2 diabetes mellitus, obesity, and cardiovascular diseases as well as biochemical abnormalities including hyperandrogenism, insulin resistance, and glucose intolerance[2-4]. While the exact cause of PCOS is still unclear, insulin resistance and hyperandrogenism may contribute to the pathogenesis of PCOS[5]. Previous studies have reported that insulin resistance plays a role in the inflammation process[6&7]. Interestingly, some studies have shown the development of chronic low-grade inflammation among women with PCOS through an increase in some markers of inflammation such as C-reactive protein (CRP) and proinflammatory cytokines among women with PCOS[8&9]. However, the definite cause of the chronic low-grade inflammation is not determined. It could be related to insulin resistance, hyperandrogenism, obesity, or as a consequence of PCOS[8&10]. The current study aimed to evaluate the inflammatory status among women with PCOS in Gaza Strip by measuring the concentration of CRP and white blood cell (WBC) count in the newly diagnosed PCOS women and comparing them with the healthy controls and the PCOS-treated women.

MATERIAL AND METHODS

This study is a cross-sectional study carried out in the Gaza Strip to assess the inflammatory status in women with PCOS. Ethical approval was obtained from the

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Helsinki Committee for Research Ethics in the Ministry of Health-Palestine.

Study population

180 women selected from gynecologic private clinics in Gaza Strip have participated in this study. All participants signed an informed written consent form. The participants were divided into three groups: control group (60 healthy women); newly diagnosed PCOS group (60 newly diagnosed women with PCOS); treated PCOS group (60 women who were on 1.2 gm/day N-acetylcysteine treatment for more than 3 months). Healthy women and PCOS patients were matched according to age (20-40 years) and body mass index (BMI) which was under 28 (kg/m^2). The diagnosis of PCOS and the selection of women to be included in the study was based on Rotterdam criteria, which is the most widely used tool for the diagnosis of PCOS. According to the Rotterdam criteria, a clinical diagnosis of PCOS requires that a woman be present with at least two of the following conditions: 1) hyperandrogenism, 2) oligo or anovulation, and 3) polycystic ovary in ultrasound checkup. Regarding the control group, the subjects were selected from healthy women admitted to outpatient clinics for periodic medical examinations, and they should be with normal menstruation, without hyperandrogenism, chronic inflammation, or chronic diseases. On the other hand, women with malignant tumors, pregnancy, breastfeeding, hyperprolactinemia, diabetes mellitus, chronic inflammatory diseases, thyroid dysfunction, viral and bacterial infections, and Cushing syndrome as well as women who received hormones, oral contraceptives, or anti-inflammatory drugs within the previous three months were excluded from the study.

Laboratory tests

Peripheral blood (PB) samples were collected from both healthy volunteers and the newly diagnosed PCOS patients on the fourth day of the menstruation cycle (for women with regular menstrual cycles) or its equivalent (for women with amenorrhea). For PCOS patients who were on N-acetylcysteine treatment, the samples were collected on the fourth day of the menstruation cycle or its equivalent after 3 months of the treatment. Samples were aliquoted and cryopreserved for analysis. Serum levels of fasting blood glucose (FBS), fasting blood insulin (FBI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), C-reactive protein (CRP) as well as white blood cell count were assessed for all participants using commercially available kits and according to manufacturer's instructions.

Clinical Assessment

The morphology of ovaries was evaluated for all participants using transabdominal or transvaginal ultrasound. Moreover, BMI was calculated using the following equation: weight (kg)/height $(m)^2$.

Statistical analysis

Graphics and statistical analyses were performed using Graphpad Prism software (San Diego, CA, USA). Data will be presented as mean ± SD. Comparisons between two different groups will be performed using unpaired Student's *t*-test. For all tests, *P* values ≤ 0.05 were considered to be significant (* $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$).

RESULTS AND DISCUSSION

Results

Characteristics of the study population

Table 1 shows the anthropometric characteristics, hormonal, metabolic, and inflammatory profile of healthy individuals and PCOS patients. The data showed no statistically significant differences in age and BMI between the study populations. There were significant differences in the levels of FPG, FPI, HOMA-IR, and LH among newly diagnosed PCOS patients compared with the healthy controls and treated PCOS patients. We also found statistically significant differences in CRP concentrations and WBC count in newly diagnosed PCOS patients compared with the healthy group and treated PCOS group.

Table 1: Characteristics of the study population

Variable		Healthy Group	Newly diagnosed PCOS Group	Treated PCOS Group	P values
		N=60	N=60	N=60	
Age (year)	Mean ± SD (range)	35.50 ± 1.3 (20 – 41)	34.05 ± 1.1 (21 – 42)	35.85 ± 1 (22 – 41)	0.8¥, 0.78€
BMI (kg/m^2)	Mean ± SD	23.73 ± 1.9	24.95 ± 1.7	25.21 ± 1.4	0.53¥, 0.67€
FPG (mg/dl)	Mean ± SD	94.55 ± 0.23	97.91 ± 0.37	95.50 ± 0.23	< 0.001¥, < 0.001€
FPI (mIU/L)	Mean ± SD	5.81 ± 0.12	8.94 ± 0.17	7.11 ± 0.13	< 0.01¥, < 0.05€
HOMA-IR	Mean ± SD	1.37 ± 0.03	2.12 ± 0.04	1.57 ± 0.03	< 0.001¥, < 0.05€
LH (mIU/L)	Mean ± SD	10.07 ± 0.31	11.8 ± 0.35	10.82 ± 0.30	< 0.001¥, < 0.05€
FSH (mIU/L)	Mean ± SD	7.21 ± 0.19	6.74 ± 0.13	7.08 ± 0.13	< 0.05¥, > 0.05€
CRP (mg/L)	Mean ± SD	3.44 ± 0.16	6.21 ± 0.21	4.36 ± 0.11	< 0.001¥, < 0.01€
WBC Count (× 10^9/L)	Mean ± SD	7.1 ± 0.2	9.98 ± 0.24	8.1 ± 0.18	< 0.01¥, < 0.05€

BMI: Body mass index; FPG: Fasting plasma glucose; FPI: fasting plasma insulin; HOMA-IR: Homeostatic model assessment for insulin resistance; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; CRP: C-reactive protein; WBC: White blood cell. ¥) p-values which represent the comparison between the means of healthy control group and the newly diagnosed PCOS group. €) p-values which represent the comparison between the means of the newly diagnosed PCOS group and the treated PCOS group. All p-values were calculated by unpaired Student's *t* test.

PCOS shows changes in patients' metabolic profile

To evaluate the effect of PCOS on the metabolic profile of PCOS patients, we measured the concentrations of FPG (mg/dl) and FPI (mIU/L) in the PB samples of the newly diagnosed PCOS patients (n= 60), the treated PCOS patients (n= 60), and the healthy controls (n= 60). Our results in (Fig. 1A) showed a significant increase in the mean concentration of FPG (97.91 ± 0.37 mg/dl) in the newly diagnosed PCOS patients compared with the healthy subjects (94.55 ± 0.23 mg/dl, *p*-value < 0.001) as well as the treated PCOS patients (95.50 ± 0.23 mg/dl, *p*-value < 0.001). We also found that the mean concentration of FPI significantly increased among newly diagnosed PCOS patients (8.94 ± 0.17 mIU/L) compared with healthy volunteers (5.81 ± 0.12 mIU/L, *p*-value < 0.01) as well as treated PCOS patients (7.11 ± 0.13 mIU/L, *p*-value < 0.05), (Fig. 1B). Then we assessed the effect of PCOS on insulin resistance by calculating the HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) for all participants based on the following equation: HOMA-IR= (Glucose mg/dl * Insulin mIU/L)/405. As shown in (Fig. 1C), there was a significant increase in the mean of HOMA-IR in the newly diagnosed group (2.12 ± 0.04) compared with the control group (1.37 ± 0.03, *p*-value < 0.001), and the treated group (1.57 ± 0.03, *p*-value < 0.05). These findings suggest that PCOS can reduce insulin sensitivity among women with the disease.

PCOS shows changes in patients' hormonal profile

Results in (Fig. 2) present the effect of PCOS on the levels of LH and FSH. Here we quantified the concentrations of LH (mIU/mL) and FSH (mIU/L) in the PB samples of all participants. We found differences in mean FSH concentrations between participants (Fig. 2A) where the results revealed a statically significant decrease in FSH levels in the newly diagnosed group (6.74 ± 0.13 mIU/L) compared to the control group (7.21 ± 0.19 mIU/L, p-value < 0.05), while the difference was not significant compared to the treated group (7.08 ± 0.13 mIU/L, p-value > 0.05). Regarding the effect of PCOS on LH levels, there was a significant increase in the mean of LH concentration in the newly diagnosed group (11.8 ± 0.35 mIU/L) compared to the control group (10.07 ± 0.31 mIU/L, p-value < 0.001), and treated group (10.82 ± 0.30 mIU/L, p-value < 0.05) (Fig. 2B). The results showed there was no significant difference in the levels of FSH (p-value = 0.57) and LH (p-value = 0.09) between the control group and the treated group, respectively. These results propose that women with PCOS may have changes in the hormonal profile in the context of FSH and LH.

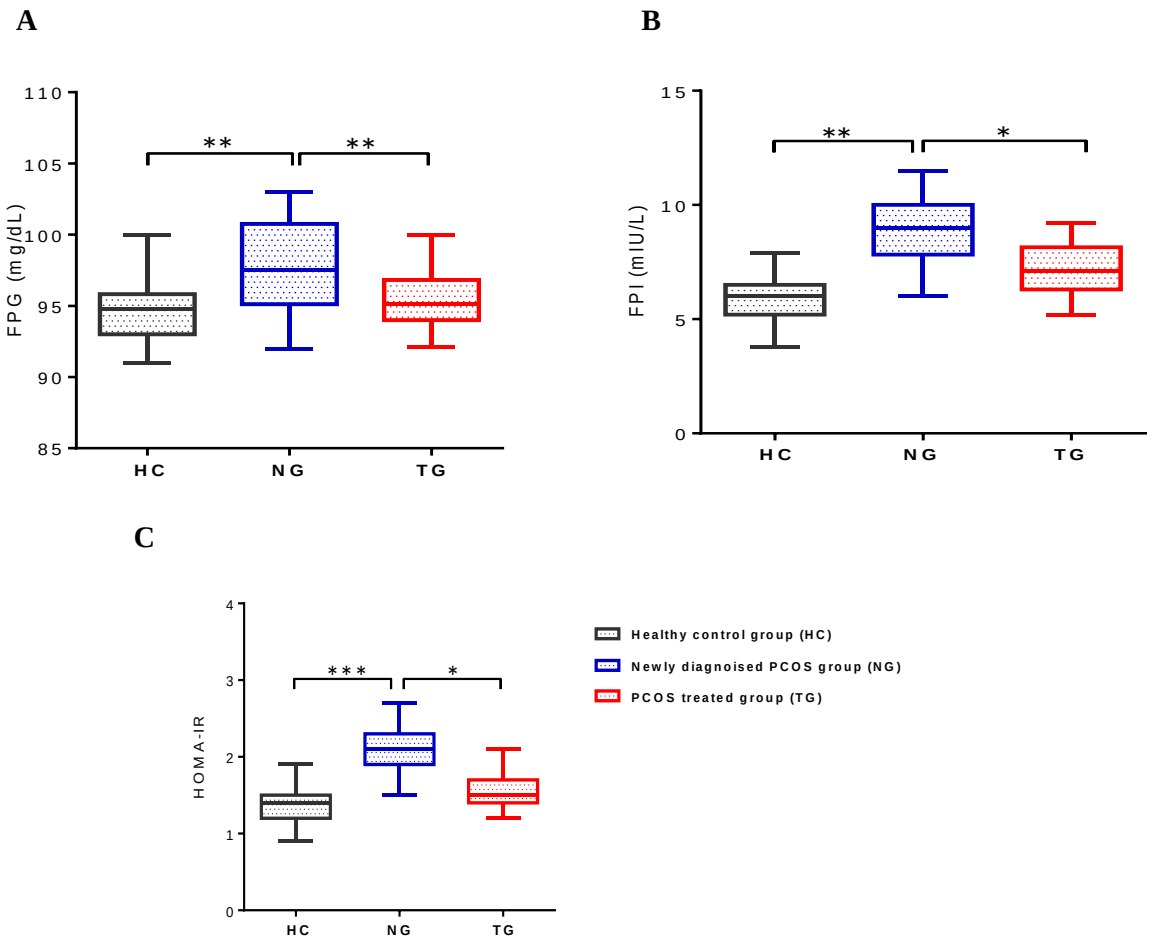


Fig. 1: PCOS shows changes in patients' metabolic profile. PB samples were obtained from all participants to measure the concentrations of FPG (mg/dL) and FPI (mIU/L). HOMA-IR was calculated to assess the insulin sensitivity among the participants. (A-C) Box and whisker graphs displaying the concentrations of FPG (mg/dL) and FPI (mIU/L) as well as HOMA-IR values in PB of healthy controls (n=60, black boxes), newly diagnosed PCOS group (n=60, blue boxes), and treated PCOS group (n= 60, red boxes). Statistical analysis was performed using unpaired Student t-test (*p ≤ 0.05, ** p < 0.01, *** p < 0.001).

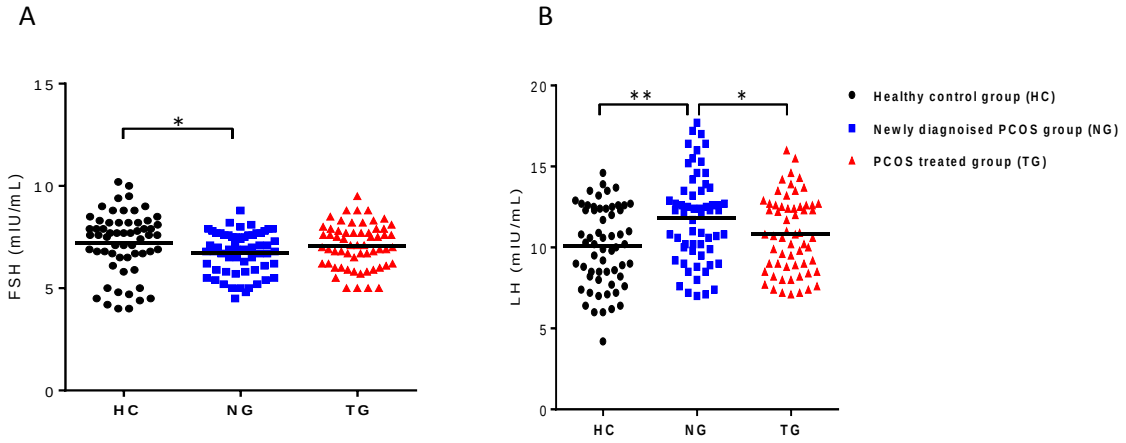
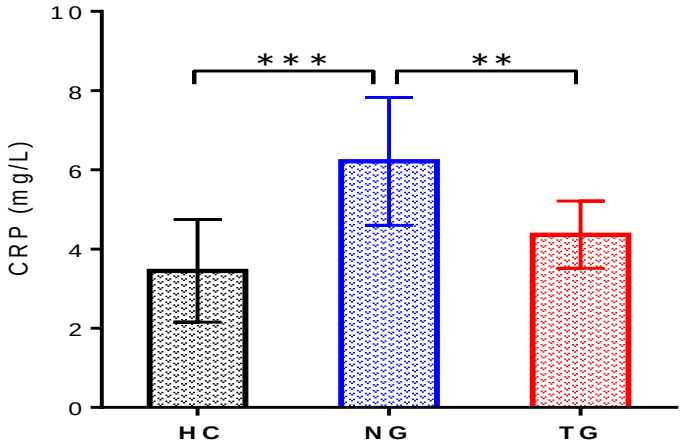


Fig. 2: PCOS shows changes in patients' hormonal profile. PB samples were obtained from all participants to measure the concentrations of FSH (mIU/L) and LH (mIU/L). A and B) Scatter dot plots showing a comparison between healthy controls (n=60, black circles), newly diagnosed PCOS group (n=60, blue squares), and treated PCOS group (n= 60, red triangles) in the term of FSH and LH levels (mean ± SD). Statistical analysis was performed using unpaired Student t-test (*p ≤ 0.05, ** p < 0.01, *** p < 0.001).

PCOS is associated with an inflammatory process

To study whether PCOS stimulates the inflammatory process in PCOS women, we evaluated CRP levels and WBC count in the newly diagnosed PCOS patients, treated PCOS patients, and healthy controls. We observed significant differences in CRP concentrations in the newly diagnosed PCOS patients (6.21 ± 0.21 mg/L) compared with the healthy controls (3.44 ± 0.16 mg/L, *p*-value < 0.001) as well as the treated PCOS patients (4.36 ± 0.11 mg/L, *p*-value < 0.01) (Fig. 3A). On the other hand, there was no significant change in the CRP concentration between the control group and the treated group (*p*= 0.083). In addition, WBC count was significantly higher in the newly diagnosed PCOS group (9.98 ± 0.24×10^9/L) compared with the healthy controls (7.1 ± 0.2 × 10^9/L, *p*-value < 0.01) and the treated PCOS patients (8.1 ± 0.18×10^9/L, *p*-value < 0.05), while the was no significant change in the WBC count between the control group and the treated group (*p*= 0.13) (Fig. 3B). These findings suggest that inflammation and increased WBC count do occur in PCOS.

A



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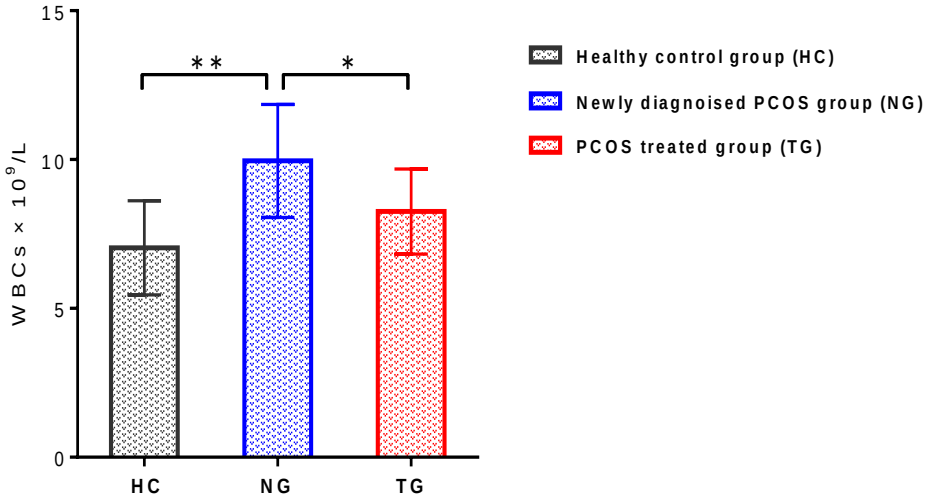

Fig. 3: PCOS is associated with an inflammatory process. PB samples were obtained from all participants to measure CRP levels (mIU/L) and WBC count (x 10^9/L). A and B) Bar diagram showing CRP concentrations and WBC count with mean ± SD in healthy controls (black bar plot), newly diagnosed PCOS group (blue bar plot), and treated PCOS group (red bar plot). Data shown here are representative of sixty (60) participants per group. Statistical analysis was performed using unpaired Student t-test (*p ≤ 0.05, ** p < 0.01, *** p < 0.001).

DISCUSSION

PCOS is a hormonal disorder that is common in reproductive age women [1&2]. In the current study, we have evaluated some metabolic and hormonal parameters as well as inflammatory status among PCOS patients in Gaza Strip. Women fulfilled the three most common PCOS diagnostic criteria for PCOS, then the participants were divided into three groups: Healthy controls, newly diagnosed PCOS patients and PCOS treated patients. Some women with PCOS showed changes in both hormonal and metabolic profiles. In our study, we found significant changes in metabolic and hormonal profiles in the context of FPG, FPI, LH, and FSH parameters in the newly diagnosed PCOS women compared to other participants. We found significant changes in the concentrations of FPI, FPG, LH, and FSH among the newly diagnosed POCS women compared with the controls and the treated group. Some previous studies also showed clear differences in hormonal and metabolic parameters between non-PCOS and PCOS women[11-14]. Since insulin resistance is common in women with PCOS and is believed to be a key factor in the pathogenesis of PCOS[15], we calculated HOMA-IR to assess the effect of PCOS on insulin sensitivity among all participants. As expected, we found a noticeable decrease in insulin sensitivity in the newly diagnosed PCOS women compared to healthy controls as well as PCOS-treated women. Our findings were similar to the results of a meta-analysis study conducted in Brazil (2021). The study found that the levels of FPG and HOMA-IR were significantly higher in PCOS Brazilian women compared to healthy controls [16]. Another study carried out in 2010 showed significant differences in insulin concentration and HOMA-IR between the PCOS group and non-PCOS controls[11]. Interestingly, we found that no valuable changes between the treated PCOS women and controls in the levels of FPI, FPG, LH and FSH, as well as HOMA-IR. Several studies also found a significant differences in the hormonal and metabolic parameters between newly dragonized PCOS patients and treated PCOS patients. For example, a study carried out by Javanmanesh et al. (2016) showed that after 24 weeks of treatment with metformin or N-acetylcysteine, FPG, FPI and HOMA-IR were significantly decreased compared to the newly diagnosed women[17]. Another study performed by Ali et al (2017) also revealed that treatment of PCOS was effective in improving the metabolic parameters and insulin resistance[18]. Together, these findings suggest that insulin resistance may participate in the pathogenesis of PCOS.

Next, we evaluated whether PCOS is associated with the development of inflammation in PCOS women by comparing the concentration of CRP and WBC count in newly diagnosed PCOS women, treated PCOS women, and healthy controls. Many studies found the development of chronic inflammation in PCOS women through increasing the levels of some inflammatory markers such as CRP and pro-inflammatory cytokines[8,9&19]. The first study to assess the association between PCOS and the inflammatory state in PCOS patients was conducted in 2001 by Kelly and coworkers[9]. They found that women with PCOS had significantly elevated CRP concentrations relative to controls. Another study showed a significant elevation in the concentrations of some proinflammatory cytokines in the PCOS group compared to the non-PCOS group[20]. A study carried out by Tarkun and his colleagues found that the serum levels of IL-6 in patients with PCOS were higher than the control group, and IL-6 was associated with insulin resistance and increased FPG[21]. Interestingly, the results of our study were in agreement with the results of previous studies regarding the development of the inflammation process among PCOS patients. We found a significant elevation in the concentration of CRP as well as WBC count among the newly diagnosed PCOS women compared to the treated PCOS women and the healthy controls. Of note, we found inconspicuous differences in CRP levels and WBC count between the healthy controls and the treated PCOS women. Some studies have linked the development of inflammation among women with PCOS to insulin resistance in these women[6,7&14]. Rudnicka et al. showed that CRP concentration, as well as WBC count, were significantly higher in the PCOS group, and both CRP level and WBC count positively correlated with serum insulin concentration [14]. In the same context, we found the development of the inflammation process in PCOS women

with insulin resistance. Altogether, these findings suggest that insulin resistance plays a key role in the development of the inflammation process among women with PCOS.

In conclusion, we suggest that PCOS is related to hormonal and metabolic changes which were clear with the development of insulin resistance among PCOS patients. Moreover, there was an increase in CRP concentration and WBC count among women with PCOS which supports the belief that PCOS is associated with inflammation. However, because various factors can reduce insulin sensitivity and affect CRP and WBC levels, additional studies are required to recognize the exact mechanism of the development of inflammation in women with PCOS.

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نشرة العلوم الصيدلية
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عنوان الحالة الالتهابية لدى النساء المصابات بمتلازمة تكيس المبايض في قطاع غزة

محمد طه

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متلازمة تكيس المبايض (PCOS) هي اضطراب هرموني شائع بين النساء في سن الإنجاب. هدفت الدراسة إلى تقييم الحالة الالتهابية لدى النساء المصابات بمتلازمة تكيس المبايض في قطاع غزة عن طريق قياس تركيز بروتين سي التفاعلي (CRP) وعدد خلايا الدم البيضاء (WBC) لدى النساء المشاركات في الدراسة. تم اختيار ١٨٠ سيدة من العيادات النسائية الخاصة في قطاع غزة وتم تقسيمهن إلى ثلاث مجموعات: مجموعة الاصحاء (ن = ٦٠). مجموعة متلازمة تكيس المبايض المشخصة حديثًا (ن = ٦٠)؛ مجموعة متلازمة تكيس المبايض المعالجة (ن = ٦٠). أظهرت نتائجنا اختلافات كبيرة في متوسط تركيزات FPG و FPI و FSH و LH في مرضى متلازمة تكيس المبايض المشخصين حديثًا مقارنة بالأشخاص الأصحاء وكذلك مرضى متلازمة تكيس المبايض. لاحظنا أيضًا زيادة كبيرة في تركيزات CRP وعدد كرات الدم البيضاء في مرضى متلازمة تكيس المبايض المشخصين حديثًا مقارنة بالأصحاء ($p<0.001$ ، $p<0.01$ على التوالي) وكذلك مرضى متلازمة تكيس المبايض المعالجين (القيمة $p<0.01$ ، $p<0.05$ على التوالي. في الختام، تشير هذه النتائج إلى أن متلازمة تكيس المبايض يمكن أن تزيد من مقاومة الأنسولين بين نساء متلازمة تكيس المبايض والتي قد تلعب دورًا في عملية الالتهاب بين نساء متلازمة تكيس المبايض.