Survey of some immunological and bacteriological parameters in Iraqi women infected with Polycystic Ovary Syndrome

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ABSTRACT

Background. Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders affecting women in their reproductive years. Obesity, an ovulation, and hyperandrogenism are linked to PCOS. Patients with PCOS exhibit increased levels of inflammatory molecules, including IL-6. An inflammatory disease called bacterial vaginosis is brought on by multiple pathogenic bacteria. Numerous recent and ongoing studies examine the variety of microbes in PCOS women's H.V.S.

The aim of this study is to identify and examine the microbium component of PCOS women's vagina. and to determine serum IL-1, IL-6, WBC, lymphocytes, and some physiological, clinical, and socio-demographic characteristics.

Methods. Sixty women in the PCOS case group and 60 healthy women in the control group of ages 18–45 were included. The study was conducted from November 10, 2023, to February 20, 2024, in hospitals in the city of Mosul (Al-Khansa Teaching Hospital and Al-Salam Teaching Hospital).

Results. Age and BMI did not show any statistically significant variations. The bacteriological aspect revealed that, of the 60 PCOS patients, 15 were Gram-positive bacterial isolates (25%) (*Staphylococcus aureus, Staphylococcus haemolyticus,*

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Streptococcus thoraltensis, and *Enerococcus faecalis*), and 45 were Gram-negative bacterial isolates (75%) (*Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Pseudomonas stutzeri*). Also, a high level of IL-1 and IL-6 was found in the serum of the PCOS group in contrast to the group of healthy controls, indicating a significant variations (P-value < 0.05), and no significant differences according to WBC or lymphocyte. Also, estradiol levels in the control group were higher than in the PCOS group.

Conclusion. This study demonstrated that the primary cause of infertility in women is polycystic ovary syndrome, a chronic low-grade inflammation. Regarding WBC, lymphocyte, age, and BMI, there is no significant variance (P>0.05). The vaginal microbiota exhibits microbial diversity, and there were significant differences in the levels of estradiol, ILL-1, and IL-6 between the PCOS patient group and the healthy control group.

Keyword: polycystic ovary syndrome, Interleukin -6, Interleukin-1, Vaginal swab

Introduction

One common endocrine disorder that affects about 10% of females in their reproductive years worldwide is called polycystic ovary syndrome, or PCOS. (Ahmed. *et al*., 2021). It is recognised as one of the most common causes of infertility, along with anovulation and hyperandrogenism (Almaeen *et al*., 2022; Altaf *et al*., 2023). PCOS has an effect on many dimensions of a woman's physical welfare, including long-term repercussions that stretch considerably outside of reproductive age (Al-Wandawy *et al*., 2020). Although the exact origin of PCOS is unknown, a number of factors have been linked to the development of metabolic and hormonal abnormalities, which may be precursors to the beginning of the condition.(Armata *et al*., 2024). PCOS is a major factor in the health of women in the future since it can lead to a number of metabolic conditions, including diabetes, obesity, and insulin resistance (IR). (Attia *et al*., 2023; Barrouq *et al*., 2023). Obesity has been linked to PCOS severity and elevated levels of TNF-α and other inflammatory factors (cytokines) in PCOS women (Barrouq *et al*.,2023). An increased rate of neutrophil production from myeloid precursor cells has been linked to androgens.

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IL-6 couples with IL-6R in the pro-inflammatory trans-signalling pathway, and IL-6R subsequently binds to gp130 in a cell membrane (Cena *et al.*, 2020; Chee *et al.*,2020). Numerous typical microbial and fungal groups, with lactobacilli as the predominant genus, colonize the vagina of a healthy female (Eiras *et al.*,2022). As vaginal bacteria are an essential component of the reproductive tract's milieu, the body gains from their homeostasis. There is mounting evidence that a woman's vaginal microbiota composition can have a major impact on her sexual and reproductive health (Giampaolino *et al.*, 2021). Also, all Gram-negative bacteria, like *E. coli,* have the most important structure in their cell walls, stimulate the immune system, and produce many cytokines such as TNF-α and IL-6 (H Mohamed *et al.*,2023). The vaginal ecosystem includes stratified squamous epithelial cells that cover the mucus layer, as well as vaginal bacteria, neutrophils, macrophages, classical dendritic cells, Langerhans cells, NK cells, T and B lymphocytes, and other innate and adaptive immune cells.

Materials and methods

Sample Collection and Processing

This study was conducted in Nineveh Governorate, 60 blood and vaginal swab samples were collected from females with polycystic ovarian syndrome in hospitals in the city of Mosul (Al-Khansa Teaching Hospital and Al-Salam Teaching Hospital). While 60 blood samples were collected from healthy women and used as a control group, without vaginal swab, for the period between November 10, 2023, and February 20, 2024. Included different age groups ranging from (18 to 45) years. 2ml of blood was placed in a tube treated with EDTA(ethylene diamine tetraacetic acid) used to perform complete blood counts (CBC). 3ml of blood was added into a gel tube to obtain serum to measure the level of IL-6. Amies transport medium was used to collect vaginal swab samples by a specialized gynecologist from the lateral posterior vaginal fornix. Freshly prepared media (nutrient agar, MacConkey agar, blood agar, and chocolate agar) were prepared for the isolation and identification of bacteria. All the isolated bacteria were identified by the VITEK-2 Compact BioMérieux.

Statistical Analysis

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SPSS (Statistical Package for the Social Sciences, version 26, USA) and Microsoft Office Excel 2010 (version 10) were used for data collection and analysis. The findings were displayed as mean ± standard deviation (m±SD). A significance level of p < 0.05 was selected for statistical analysis.

Results

A Physiological Study of PCOS

The table (1) below indicates no discernible change (P-value >0.05) in age between PCOS and control groups, with mean values of (29.11±7.14) and (31.10±6.89), respectively. PCOS patients had a BMI of (27.33±3.63 kg/m^2), while the control group had a BMI of (26.62±4.19). According to estradiol, the PCOS and control groups differ greatly from one another, with mean values of (108.66±29.41) and (193.27±38.46), respectively. Based on PCOS groupings, there were (76.67%) suspected infertile women and (23.33%) fertile women. In contrast, the control group had (0.0%) infertile women and (100%) fertile women. Between PCOS patients and the control group, there was a significant difference (P-value < 0.001). According to the results of the current study, of the PCOS patients, (31.67%) had regular menses, and (68.33%) had irregular menses (oligomenorrhea and amenorrhoea). Within the control group of the same study, (91.67%) reported regular menstruation, while (8.33%) reported irregular menstruation. Between PCOS patients and the control group, there was a significant difference (P-value < 0.05). On the other hand, in patients with polycystic ovarian syndrome, there were (66.67%) hirsutism women and (33.33%) non-hirsutism women; in the control group, there were (0.0%) hirsutism women and (100%) non-hirsutism women. Between PCOS patients and control groups differ greatly from one another (P > 0.001). The results also showed that the women with acne was (55.0%) and without acne was (45.0%), while in the control group, the women with acne was (16.67%) and women without acne were (83.33%). There was a significant difference between PCOS patients and the control group (P< 0.05). Women with PCOS frequently experience stress and anxiety, yet these conditions are frequently disregarded and go untreated. In the present study for PCOS patients was (90%) based on questionnaire form and non-stress was (10%), while the control group have stress was (16.67%) and non-stress was (83.33%). There was a significant difference between PCOS patients and the control group (P > 0.05). Table 1 below also shows that the education

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level of PCOS patients in the current study was (60%),(23.33%), and(16.67%)} for primary school, secondary school, and graduation, respectively, as compared to the control group of (11.67%),(30%),and (58.33%). The results of our study also showed, the housewives were (75.0%), and employers were (25.0%), while in the control group, the housewives were (65.0%), whereas the employers were (35.0%). There was no significant difference between PCOS patients and the control group (P-value <0.05). Regarding metformin treatment, the current study showed that 91.67% of PCOS women and 8.33% of the healthy control group used metformin treatment. There was a significant difference between PCOS patients and the control group (P > 0.05).

Immunological study

The level of concentration of IL-1 and IL-6 in the group of patients with PCOS compared to the healthy control group was estimated in the serum of the studied sample by using the ELISA technique , WBC and lymphocytes by the Sysmex device, as shown in Table 2 below. Statistical analysis for level concentration of immunological parameters (IL-6, IL-1) in patients with PCOS compared to a healthy control group using an independent t-test (two-tailed) and a Mann-Whitney, as shown in Table 4 below, high level of IL-6 and IL-1 found in the serum of the PCOS group compared with the healthy control group, and a significant difference (P-value < 0.05). When comparing the mean levels of IL-6 and IL-1 between the PCOS patient group and the healthy control group, it was found that the former had a mean of 7.030±5.76 and the latter had a mean of 5.041±1.17 pg/ml. With reference to the WBC, Furthermore, in this study, there was no statistically significant difference (P >0.05) in the white blood cell (WBC) counts of PCOS patients and healthy. Both PCOS cases and healthy controls had WBC means and SDs of 7.07±2.18% and 6.69±1.88%, respectively. In terms of lymphocyte count, the mean and standard deviation of lymphocytes were 34.09±12.83 in PCOS cases and 34.79±10.13 in healthy controls. Nonetheless, there was no significant correlation (P > 0.05) found between the control group and PCOS patients.

Bacteriological study

According to Table 3 below, the bacteriological aspect revealed that, of the 60 PCOS patients, 15 were gram-positive bacterial isolates (25%), and 45 were gram-negative bacterial isolates (75%). The result showed that the vaginal infection was more frequent

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in women between the ages of 27 and 35) years old. According to age groupings, the age group (27–35) years old had the highest prevalence of vaginal infection with bacteria among patients (26; 43.34%), 4 patients (6.67%) for gram-positive bacterial isolates, and 22 patients (36.67%) for gram-negative bacterial isolates. The results in Table 4 below showed that most PCOS women infected with *Staphylococcus spp. (n = 11*; 18.33%) and *E. coli* (n = 22; 36.67%%) were the most common isolates of PCOS women. According to the results of our investigation, bacterial vaginal infections were present in every woman with polycystic ovarian syndrome. Percentage Of Gram-Positive Bacterial Species, Gram-positive isolates were represented by *Staphylococcus aureus* 5/14(%) and *Staphylococcus haemolyticus* also accounted for 6/15(%), they were predominant isolate followed by *Enerococcus faecalis* 3/15(%) and *streptococcus thoraltensis* 1/15(%).Percentage Of Gram-Negative Bacterial Species, Gram-negative bacterial isolate were represented by *K. pneumonia* 20/45(%),*E. coli* 22/45(%), *Pseudomonas stutzeri* 1/45(%) and *Proteus mirabilis* 2/45(%).

Table (1): Illustrate The Basic Clinical And Socio-Demographic Characteristics Of Study Group

Variables		PCOS women N=60		Control group N =60		*P* value
		Count	%	Count	%	
History of infertility	Infertility	46	(76.67%)	0	(0%)	P<0.001*
	Fertility	14	(23.33%)	60	(100%)	
Menstrual cycle	Irregular	41	(68.33%)	5	(8.33)	P<0.05
	Regular	19	(31.67)	55	(91.67%)	
Hirsutism	Yes	40	66.67%	0	(0%)	P<0.001*
	No	20	33.33	60	100%	
Acne	Yes	33	%55	10	16.67%	P<0.05
	No	27	%45	50	83.33	
Stress	Yes	54	(90%)	10	16.67%	(P<0.05)
	No	6	10%	50	83.33%	
	Primary school	36	(60%)	7	(11.67)	P<0.05

Educational level	Secondary school	14	(23.33%)	18	(30%)	
	Graduated	10	(16.67)	35	(58.33%)	
Occupation	Housewife	45	(75%)	39	65%	P>0.05
	Employer	15	(25%)	21	35%	
Metformin	Yes	55	91.67%	0	0%	P<0.05
	No	5	8.33%	60	100%	

N =number ,PCOS=polycystic ovary syndrome ,P<0.05 means significant variance ,P>0.05 means no significant variance .

Table (2): IllustrateIL-1, IL-6, WBC, lymphocyte, age, BMI and E2 in PCOS women and non- PCOS women

Parameter	Patients with PCOS		Control Group	P-value
	N	**(Mean ±SD)**	**(Mean ±SD)**	
IL-6	60	7.030±5.76	5.041±1.17	P<0.05
IL-1	60	29.90±21.25	19.67±8.70	P<0.05
WBC	60	7.07±2.18	6.69±1.88	P>0.05
Lymphocyte	60	34.09±12.83	34.79±10.13	P>0.05
Age	60	29.11±7.14	31.1±6.89	P>0.05
BMI	60	27.33±3.63	26.62±4.19	P>0.05
Estradiol (E2)	60	108.66±29.41	193.27±38.46	P<0.05

N= number, means significance differences (P <0.05), SD means standard deviation, IL-1 means interleukin 1,IL-6 means interleukin 6, BMI means Body Mass Index.

Table (3): Distribution of gram positive and gram negative bacterial isolate according to age and their percentage.

Ages (years)	Bacterial growth				Total	
	G + ve		G -ve			
	NO.	%	NO.	%	NO.	%
18-26	5	8.33%	19	31.66%	24	**39.99%**
27-35	4	6.67%	22	36.67%	26	**43.34%**
36-45	6	10%	4	6.67%	10	**16.67%**

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Total	15	25%	45	75%	60	100%

No. means number, G- means Gram negative bacteria ,G+ means Gram positive bacteria, % means percentage.

Table 4 shows the percentage of bacterial species.

Types of bacterial isolates	PCOS women with HVS infection	
	No.	**%**
Staphylococcus aureus	5	8.33%
Staphylococcus haemolyticus	6	10%
Streptococcus thoraltensis	1	1.66%
Enerococcus faecalis	3	5%
Escherichia coli	22	36.67%
Klebsiella pneumoniae	20	33.33
Proteus mirabilis	2	3.33
Pseudomonas stutzeri	1	1.67
Total	60	100%

No. means count of isolate , HVS means high vaginal swab, PCOS means polycystic ovary syndrome.

Discussion

About 10% of females in their reproductive years worldwide suffer with PCOS, a common endocrine disorder. It is recognized as one of the most typical causes of infertility. As show in Table (1) there is no significant difference (P-value >0.05) in age between PCOS and control groups. This difference was not statistically significant. The current findings were consistent with (Hoeger *et al.*, 2021), as well as (Giampaolino *et al.*, 2021). Iraqi women with PCOS have a higher upper body weight than women with the disease in other countries, which may be related to the different food habits in our country and the low level of physical activity among Iraqi women. A healthier lifestyle can help PCOS patients who gain weight because studies have shown that this lowers testosterone levels, which raise insulin sensitivity, and improves hormonal health. (Hong *et al.*, 2021).

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Additionally, according to estradiol (E2) there was significant differences (P-value <0.05) were found between the PCOS patients and the control group. The present findings are consistent with the research conducted by (Hong *et al.*, 2020). And disagree with the result of (Ibraheem *et al.*, 2022). This differences in the result due to the condition or the state of patient cases because the sample collected from patient who under treatment with metformin that causes to reduce the estrogen level in patient.

Based on PCOS groupings, there were (76.67%) suspected infertile women and (23.33%) fertile women. In contrast, the control group had (0.0%) infertile women and (100%) fertile women, there was a significant difference (P-value < 0.001). This outcome was consistent with research conducted by (Jassim *et al.*, 2020).The current study included only married, fertile, and healthy women as control participants (Joham *et al.*, 2022). Regarding regular and irregular menses between PCOS patients and the control group, there was a significant difference (P-value < 0.05). The results of this study are consistent with (khadheir Zaghir *et al.*, 2023).

On the other hand, there was a significant difference between hirsutism women and non-hirsutism women (P<0.001). These findings were in accordance with a number of investigations, including one by (Kim *et al.,* 2019).A common ailment among women, hirsutism is a key clinical characteristic of polycystic ovarian syndrome (PCOS). Hirsutism is considered to be a result either of increased testosterone production or increased follicular androgen sensitivity (Mancini *et al.*, 2021).

Regarding women with and without acne, there was a significant difference between PCOS patients and control group (P<0.05). The result of this study agreement with the result of the study by (Mazloomi *et al.*, 2023). And disagree with the study by (Mendes *et al.*, 2023), the differences in these results may be due to the small volume of samples in this study. The study also found that women with PCOS had significantly higher stress levels compared to those without PCOS (P<0.05). The result of our study agreed with study of (Mohamed *et al.*, 2020), that demonstrated that there is significant differences between PCOS group and control group.

The women may have high levels of stress and anxiety for a variety of reasons, including hirsutism, alopecia, infertility, and obesity, which is the main factor elevating mental stress levels.

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Table (1) shows that the education level of PCOS patients in the current study was {(60%), (23.33%) and (16.67%)} for primary school, secondary school, and graduated, respectively, as compared to the control group {(11.67%), (30%) and (58.33%)}.

The study's findings agreed with those of (Moosa *et al.*, 2020) and disagree with (Mazloomi *et al.*, 2023). This difference in results is due to differences in social communities, as well as the country's economic conditions and wars.

Regarding the housewives and employers the findings of our research revealed, the PCOS patient group did not significantly differ from the control group (P-value >0.05). These result agreed with several studies, such as a study by (Mukherjee *et al.*, 2023). This difference in results is due to differences in social communities, as well as the country's economic and educational conditions and wars.

Regarding to Metformin treatment, the current study showed that 91.67% of PCOS women and 8.33% healthy control group were used metaformin treatment. For overweight or obese patients with PCOS, metformin is advised as a first line of treatment, especially if oral contraceptives are not an option or there is obvious insulin resistance (Nandhini *et al.*, 2022). Metformin is an effective treatment for anovulation in women with polycystic ovary syndrome (Naqid *et al.*, 2020).

The results of immunological parameters presented in Table (2) above, illustrated the level concentration of IL-6, IL-1 in group of patients with PCOS compared to healthy control group. Statistical analysis for level concentration of immunological parameter (IL-6,IL-1) in patients with PCOS compared to healthy control group, using independent t-test (two tailed) and Mann-Whitney *U* test as shown in Table (2) mentioned above, show high level of IL-6, IL-1 in serum of PCOS group compared with healthy control group, significant difference (P-value < 0.05). The results of our study agreement with previous studies in close proportions such as local and international studies. All cell types express IL-6, a pleiotropic signalling inflammatory marker (Nowak et al., 2024). This cytokine affects the development of the foetus, the production of sex hormones, and the activity of the corpus luteum. IL-1, another inflammatory cytokine, is crucial for ovulation and fertilisation. Numerous studies attest to the elevated levels of IL-1 and its receptors in PCOS. (Patel *et al.*, 2017). Furthermore, when comparing healthy obese women to healthy lean ones, the concentration of IL-1 increased; however, this increase did not show up when comparing obese and lean PCOS women. Patients with PCOS who have IR have higher serum concentrations of IL-6. According to a study, PCOS women with

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infertility have higher levels of IL-6 than do healthy controls, which can have negative effects. (Patel *et al*., 2017). IR and androgens are associated with elevated IL-6 levels, but not with. The results of our investigation closely align with those of previous studies, including both domestic and foreign studies like (Joham *et al*., 2022). Thus, PCOS patients are at higher risk of insulin resistance, metabolic syndrome, obesity, and chronic inflammation(Rostamtabar *et al*., 2021).

Regarding to WBC, there was no significant association (*P*>0.05) between PCOS patients compared and healthy control. This result agreed with some studies, such as a study by(Sengupta *et al*., 2024). On the contrary, a study by (Shabbir *et al*., 2023), and in a study by (Smith *et al*., 2017). In a large national cohort study, WBC was a predictor of coronary heart disease mortality independent of smoking and other traditional risk factors (Torcia *et al*., 2009).

Regarding lymphocyte count, the association was non-significant (*P*>0.05) between PCOS patients and control groups. The current study result is compatible with a study by(Tu *et al*., 2020), on the contrary, a study by(Yasmin *et al.,* 2022).

The results in Table (4) below showed that most PCOS women infected with *Staphylococcus. spp* (18.33%) and *E. coli* (36.67%) were the most common isolates PCOS women. According to the results of our investigation, bacterial vaginal infections were present in every woman with polycystic ovarian syndrome. The current investigation of PCOS patients vaginal reveals that these patients have more diverse microbiomes (65,66%). Our study's results did not support the findings of that study, which suggested that the PCOS group in that study had a suitable environment for the growth and multiplication of *Candida. spp* due to insulin resistance or glucose tolerance brought on by PCOS pathology, these results reflect the huge variation of the vaginal inhabitants and disruption of vaginal flora in PCOS group.

The reason that makes *S.aureus* to be more invasive among Gram-positive organisms that leads to vaginal infection can be ascribed to the virulence factors, which have been characterized: protein A, collagen binding protein, fibronectin binding proteins, and the fibrinogen binding protein (Ibraheem *et al*., 2022).

Coagulase negative staphylococci (CoNS) appear to be the major pathogen worldwide and associated with significant UTI and HVI, the reason of high rate of CoNS isolates may be related to the use of broad spectrum antibiotics and to the role of specific adhesion and slime produced by CoNS(Yuxin *et al*., 2021).

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Gram-negative bacterial isolates like *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas stutzeri* were the most common pathogen present in UTI and HVI in PCOS women that agreeing with(Zhao *et al*., 2020) respectively, who confirm in their studies that the gram-negative bacterial isolates were the most prevalent bacterial infections in females because they are more susceptible to UTIs and bacterial vaginal infection than males due to anatomical differences, such as a shorter urethra and perineal contamination of the urinary tract with fecal microorganisms.

Conclusion

There is no significant variance (P>0.05), regarding Age and BMI in PCOS and control group. There is microbial diversity in the vaginal microbiota. Incidence with Gram-negative bacteria were predominant more than Gram-positive bacteria in PCOS patients especially *Staphylococcus*. spp and *E. coli* in PCOS patients in Mosul City Hospitals. Estradiol, ILL-1and IL-6 level was significantly different in group of patients with PCOS compared to healthy control group. No significantly different in group of patients with PCOS compared to healthy control group regarding to WBC and lymphocyte.

Conflict of interest

none declared

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Ethics of Study

Informed consent was obtained from each participant. The purpose and significance of the study were explained to the women. The privacy of any information gathered was guaranteed.

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