The Relationship of *Staphylococcus aureus* Virulancity with Serum Cytokine Levels in Polycystic Ovary Syndrome and Polycystic Ovary Disease

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**Abstract.** The current study was done to isolation and indentification of the *Staphylococcus aureus* isolates associated with polycystic ovarian syndrome patients and determine the immunological status for these patients. Inflammatory markers have been linked to polycystic ovary syndrome (PCOS). Because ovulation is a quasi-inflammatory process, endothelial adipose tissue can stimulate an inflammatory response and maintain inflammation in adipocytes. By influencing the release of inflammatory cytokines from this downregulated inflammation, polycystic ovary syndrome can arise. The study included 105 clinical specimens, that include age (20-45) years which were collected from November 2022 to June 2023. The specimens included blood specimens and vaginal swabs. They visit Imam Al-Sadiq Hospital and Babylon Teaching Hospital for Maternity and Children in Babylon Governorate. The results of vaginal swabs culture, biochemical tests and 2 for isolated bacteria from patients were revealed that Gram-positive bacteria isolates were dominant, amounting to 51 (54.83%), while the percentage of Gram-negative bacteria was 37 (39.78%), while fungi percentage was 5 (5.37%). The results showed that *Staphylococcus aureus* is the most common bacteria among Gram-positive bacteria, as it constituted 38 (40.86%), followed by Lactobacillus spp. As it accounted for 11 (11.82%) of *Staphylococcus epidermidis* by 2 (2.15%). The Gram-negative isolates included Escherichiae coli, Klebsiella pneumoniae, and Pseudomonase aerogenosa 23 (24.73%), 9(9.67%) and 5 (5.37%), respectively also the result demonstrates that IL-10, IL-13 level significantly elevation in PCOS, PCOD patients group.

1 Introduction

Polycystic ovary syndrome (PCOS) is characterized by the unregulated menstrual cycle (oligomenorrhea or amenorrhea), elevated androgenic hormones levels (hyperandrogenism) and many cysts of the ovaries (polycystic ovaries). Other features include male pattern
hirsutism, acne, increased skin pigmentation with tags sometimes, and obesity [1]. PCOS comorbid with insulin resistance, dyslipidemia, and obesity, it also carries significant risk for the development of cardiovascular and metabolic sequelae, including diabetes and metabolic syndrome [2]. The existence of a fully developed hypothalamic – pituitary - ovarian axis and highly harmonized hormonal feedback circles are essential for the normal ovulatory menstrual cycle. Which consisting of three phases (follicular phase, ovulatory phase, and luteal phase), the normal menstrual cycle leads to the format of a mature follicle and liberate of an oocyte during each cycle, without fertilization menses would occur [3]. Study found the most common causative organism in Polycystic ovary syndrome (PCOS) is Staphylococcus aureus but Escherichia coli, Pseudomonas aeruginosa and Staphylococcus epidermidis may also cause this infection, other gram-negative rods including Klebsiella pneumoniae, Enterobacter spp and Candida spp. which also present in Polycystic ovary syndrome (PCOS) [4]. The present of this organism with its virulence factors such as (pili, capsule, and toxins) due to hormonal disorders that make the walls of urinary tract drier and the mucous membrane less acidic, which reduces their ability to fight infection [5]. The main causes and symptoms of PCOS are thought to be hormonal imbalance and hyperandrogenism [6] Hormones and immune cells appear to play an important role in the progression of PCOS, according to growing evidence. Hyperandrogenemia can stimulate monocyte infiltration in the ovary and increase inflammatory factor secretion, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), resulting in chronic ovarian tissue inflammation, affecting the maturation of developing follicles in the ovary, and leading to cystic follicle formation. Furthermore, studies have shown that in comparison to normal women, the levels of inflammatory elements such as C-reactive protein (CRP), interleukin-1β (IL-1β), interleukin-17 (IL-17), interleukin-18 (IL-18), TNF-α, monocyte chemoattractant protein-1 (MCP1), soluble endothelial leukocyte adhesion molecule (SE selectin), and soluble intercellular adhesion molecule (sICAM) in peripheral blood of most PCOS women were considerably higher [7].

The low-grade chronic inflammation in PCOS patients is mainly attributed to accumulated visceral fat, in which adipocytes undergo necrosis after hypoxia and gather a large number of inflammatory cells to produce a variety of inflammatory cytokines. Immune cells can cause or suppress host inflammation by secreting pro-inflammatory or inhibitory cytokines. Antibodies, complements, and lymphokines are immune molecules that are produced by immune cells in response to antigen stimulation. Abnormal immune cell activity can lead to immune cell dysfunction or an imbalance in immune-related factors [5].

2 Materials and Methods

2.1 Collection of specimens

The study included 105 clinical specimens, which were collected from November 2022 to June 2023. The specimens included (105) blood specimens – (105) vaginal swabs. About 75 specimens were collected from women with polycystic ovarian syndrome (PCOS) and 30 specimens were collected from women with polycystic ovarian disease (PCOD). They visit Imam Al-Sadiq Hospital and Babylon Teaching Hospital for Maternity and Children in Babylon Governorate. While 50 specimens were collected from women without polycystic ovaries as a healthy group.
2.2 Vaginal swaps and blood specimens

The specimens were transported quickly to the bacteriology laboratory in public health laboratory and each specimen was inoculated using direct method of inoculation on different media such as MacConkey agar and Blood agar, incubated at 37°C for 24-48 hours, [8]. While The blood was collected by using sterile syringe 5ml, then transfer to tube without anticoagulant. The serum was separated by centrifugation at about 3000 rpm for 5min within 2-3 hours after collection [9].

2.3 Laboratory Diagnosis, Bacterial Identification Assays

According to the diagnostic procedures recommended by [10, 11, 12] and the isolation and identification of Gram-Negative Bacteria and Gram-Positive Bacteria that associated with patients under study were performed as follow:

2.4 Microscopic Examination and Colonial Morphology

A single colony was taken from each primary positive culture and its identification depended on the morphology properties (Colony size, shape, color, type of pigments, translucency, edge, elevation, and texture). After staining the bacteria by gram stains, specific biochemical tests were done to reach the final identification include (Catalase Test, Oxidase Test, Coagulase Test, Indol Test, Vogus-Proskaur Test, Simmons' Citrate Test, Urease Test, Motility Test, capsule Test) [13].

2.5 Vitek 2 system for Identification

The Vitek-2 system was used to confirm the biochemical test according to the manufacturer's instructions.(bioMérieux)

2.6 Immunological assay

Estimation of (IL-10, IL-13) concentration by ELISA assay
This test was conducted in accordance with the manufacturer's instructions (BT LAB).

3 Results and Discussion

3.1 Clinical study

The study included 105 clinical specimens, that include age (20-45) years which were collected from November 2022 to June 2023. The specimens included(105) blood specimens and (105) vaginal swabs. They visit Imam Al-Sadiq Hospital and Babylon Teaching Hospital for Maternity and Children in Babylon Governorate. The study revealed that 32 (30.47 %) of patients were at the age group of (20-25) years, that 26 (24.76 %) of patients were at the age group of (31-35) years, that 19 (18.09 %) of patients were at the age group of (26-30) years, that 15 (14.28 %) of patients were at the age group of (36-40) years and than that 13 (12.38 %) of patients were at the age group of (41-45) years as shown in table [1].
Table 1. Distribution of patients according to age groups and Bacteria Isolate.

<table>
<thead>
<tr>
<th>Age</th>
<th>PCOS patients N(%)</th>
<th>PCOD patients N(%)</th>
<th>Bacteria Isolate N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25 year</td>
<td>21(28.00)</td>
<td>11(36.66)</td>
<td>32 (30.47)</td>
</tr>
<tr>
<td>26-30 year</td>
<td>13 (17.33)</td>
<td>6(20.00)</td>
<td>19 (18.09)</td>
</tr>
<tr>
<td>31-35 year</td>
<td>19 (25.33)</td>
<td>7(23.33)</td>
<td>26 (24.76)</td>
</tr>
<tr>
<td>36-40 year</td>
<td>12(16.00)</td>
<td>3(10.00)</td>
<td>15 (14.28)</td>
</tr>
<tr>
<td>41-45 year</td>
<td>10(13.33)</td>
<td>3(10.00)</td>
<td>13 (12.38)</td>
</tr>
<tr>
<td>Total</td>
<td>75 (100)</td>
<td>30(100)</td>
<td>105(100)</td>
</tr>
</tbody>
</table>

This result is in agreement with [14] and not agreement with [15], [16] and [17]. The similarities between these studies regarding the same age group because PCOS appeared at menarche and the women became symptomatic later but most women with PCOS are diagnosed when their age between 20-30 years [18]. The estimated prevalence of PCOS among women of reproductive age exhibits significant variation across the world, with reported rates ranging from 4% to 20%. Such variation can be attributed to differences in diagnostic criteria, the characteristics of the population under study, and the diagnostic methods employed.

3.2 Vaginal swabs culture

The present study included the collection of 105 vaginal swabs. specimens collected from women with polycystic ovarian syndrome (PCOS) and specimens were collected from women with polycystic ovarian disease (PCOD). Morphological and biochemical characterization indicated that 93 (88.57%) of specimens had positive bacterial culture and 12 (11.42%) of them had negative culture.

The results of vaginal swabs culture, biochemical tests and vitic2 for isolated bacteria from patients were revealed that Gram-positive bacteria isolates were dominant, amounting to 51 (54.83%), while the percentage of Gram-negative bacteria was 37 (39.78%), while fungi percentage was 5 (5.37%). The results showed that *Staphylococcus aureus* is the most common bacteria among Gram-positive bacteria, as it constituted 38 (40.86%), followed by *Lactobacillus spp*. As it accounted for 11 (11.82%) of *Staphylococcus epidermidis* by 2 (2.15%). The Gram-negative isolates included *Escherichiae coli*, *Klebsiella pneumoniae*, and *Pseudomonase aerogenosa* 23 (24.73%), 9(9.67%) and 5 (5.37%), respectively. as shown in figure (3) below.
In these results many kinds of opportunistic pathogens were enriched in PCOS group, including *Staphylococcus aureus*, *Lactobacillus spp*, *Staphylococcus epidermidis* *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aerogenosa* which was in accordance with the findings of [19]. Based on previous studies, these pathogens could be associated with metabolic disorders and severe inflammation. In particular, *Staphylococcus aureus* which could be associated with another pathogen we found to be enriched in PCOS patients, has been associated with inflammation in urinary tract infections [20]. The different properties and metabolic functions of these pathogens possibly make the host more susceptible to metabolic disorders and inflammation, thus contributing to the development of PCOS. Different bacterial communities and properties might function distinctively in PCOS patients, when made a correlation with hormones. was found that the were positively correlated with hormones accordingly,Disturbances estrogen in women who suffer from ovarian syndrome makes the walls of the urinary tract drier, and the mucous membrane or mucous layer becomes less acidic, which reduces its ability to fight infection previous research conducted by [19] revealed a positive correlation among Bacteroides, testosterone.

### 3.3 Immunological study

**Estimation of interleukin 10 (IL-10)**

Comparison between IL-10 level in the study groups revealed in Table (2) and Figure (3.8). the result demonstrates that IL-10 level significantly elevation in PCOS,PCOD patients group (P <0.0001) when compared with healthy control group.
Table 2. Comparison of serum interleukin 10 level between PCOS, PCOD patients group and healthy control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCOS Group Mean ± SD</th>
<th>PCOD Group Mean ± SD</th>
<th>Control group Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 (pg/mL)</td>
<td>309.0±131.8</td>
<td>137.9±44.93</td>
<td>81.37±15.61</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Fig. 4. Difference in mean levels of IL-10 between PCOS, PCOD patients with healthy control group.

3.4 Estimation of interleukin 13 (IL-13)

Comparison between IL-13 level in the study groups revealed in Table (3) and Figure (3.9), the result demonstrates that IL-13 level significantly elevation in PCOS, PCOD patients group (P <0.005) when compared with healthy control group.

Table 3. Comparison of serum interleukin 13 level between PCOS, PCOD patients group and healthy control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCOS group Mean ± SD</th>
<th>PCOD group Mean ± SD</th>
<th>Control group Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13 (pg/mL)</td>
<td>26.81±10.15</td>
<td>28.20±5.749</td>
<td>23.06±5.00</td>
<td>0.005</td>
</tr>
</tbody>
</table>
The current result revealed a significant higher level of IL-10, IL-13 in women with PCOS, PCOD when compared with healthy control women may be related to chronic high-grade inflammation, which may be suggested as a key contributor of the pathogenesis and development of polycystic ovary syndrome (PCOS). The current result agrees with [21]). The increase in IL-10, IL-13 secretion was substantially linked with PCOS illness Ovarian dysfunction, delayed follicular maturation, and altered steroidogenesis are all caused by an imbalance of pro- and anti-inflammatory cytokines [22]. Some studies have hypothesized that IL-10 may be crucial for maintaining pregnancy by supporting the formation of Th2 cytokine environment, progesterone production, and corpus luteum maturation [23]. The main characteristics of the metabolic abnormalities typical of PCOS include insulin resistance and hyperinsulinemia. There is growing proof that IL-6, IL-13, IL-10, and TNF- are crucial in triggering insulin resistance, however; The current result not agree with Some studies have low IL-10 levels have been reported to be associated with obesity and metabolic syndrome [24]. [25] found that a low level of IL-10 is related to metabolic syndrome and obesity. Reduction in plasma IL-10 was seen in PCOS patients [25].

4 Conclusion

According to the current study Polycystic ovary syndrome and Polycystic ovary disease that present is Staphylococcus aureus represented the most common bacteria isolated for patients with Polycystic ovary syndrome and Polycystic ovary disease. And also appeared increase in the level of cytokines (IL-10 and IL-13).
References


