The Polycystic Ovary Syndrome as A Cause of Increase in Inflammatory Markers and Metabolic Risks

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ABSTRACT:

BACKGROUND:
Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorder affecting women in reproductive age. No single etiologic factor fully accounts for the spectrum of abnormalities in the polycystic ovary syndrome. Different changes in hormonal, metabolism and the inflammatory markers as squealy of PCOS with adverse effect on the women life.

OBJECTIVE:
To study the relationship between polycystic ovary syndrome and levels of C-reactive protein, human interleukin and hormonal and metabolic alteration in women with PCOS

PATIENTS AND METHODS:
Thirty women with Polycystic Ovary syndrome (PCOS) and other thirty women without PCOS were included. Venous blood samples were taken in early follicular phase of menstrual cycle [day 2-6] for luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. Serum level of Human Interleukin 1B (IL-1B) was quantitatively determined. Blood samples were also tested for detection of CRP (C-reactive protein, in addition to levels of cholesterol, triglycerides.

RESULTS:
The mean cholesterol level was significantly higher in PCOS group rather than controls; (188.3 ± 7.2) vs. (168.7 ± 6.1) respectively. Mean LH level was higher in PCOS women than control (13.3 ± 2.4 IU/L) vs. (5.85 ± 1.47) IU/L, while in contrary mean FSH level was lower in PCOS cases. The mean serum testosterone in PCOS group was significantly higher than in controls, with no significant difference regarding IL-1B, but again it was significant in cases of CRP.

CONCLUSION:
PCOS was associated with higher levels of cholesterol, LH, and serum testosterone with lower levels of FSH. Positive C-Reactive Protein was more frequent in PCOS cases. Still serum level of Human Interleukin 1B (IL-1B) was not significantly affected by PCOS.

KEY WORDS: polycystic ovary syndrome, C-reactive protein, human interleukin.

INTRODUCTION:
Polycystic ovary syndrome is the most frequent cause of endocrinopathy problems affecting reproductive aged women, such as hyperandrogenism and oligo-anovulation with prevalence of (4-12%) in united states, and (6.5-8%) in European studies (1). It is associated with anovulatory infertility in up to (~75%) in infertile women (2-3). It was first described in 1935 by stein and leventhal as a frequent cause of irregular ovulation or anovulation in women seeking treatment for sub fertility. (4)

It is a heterogeneous syndrome, with multiple cosmetic, reproductive, and metabolic disorders which is characterized by a defect in ovulation with clinical or biochemical hyperandrogenism, and the presence of polycystic ovarian morphology. It is a recognized cause of infertility and increased the risk of adverse pregnancy fate, metabolic syndrome, type 2 diabetes mellitus, and some types of carcinoma. Diagnosis is based on the National Institute of Health (NIH) criteria or upon Rotterdam criteria, where there is no consensus up to date. (5,6)

One study showed a 43% prevalence of metabolic syndrome in woman with PCOS, who also have increased prevalence of coronary artery calcification and a thickened carotid intima media, which may be responsible for sub clinical atherosclerosis (7).
The majority of women with PCOS have anovulation. With this comes infertility as well as problems of dysfunctional bleeding. Chronic unopposed estrogen leads to endometrial hyperplasia and, potentially, to cancer. Because 20% of women may have polycystic appearing ovaries on ultrasound, of which one out of four will have PCOS, ultrasonography is not a specific test. Accordingly, a woman may have features of PCOS without signs of hyperandrogenism and, in that context, measurement of androgens may contribute to the diagnosis. In such a case, serum-free testosterone or free androgen index are the best tests to eliminate changes of sex hormone-binding globulin. PCOS is a common problem in reproductive-age women. It can present from adolescence to menopause. The physician should recognize that a withdrawal bleed at least every three months is needed to prevent the theoretic increased risk of endometrial carcinoma. Fertility may be normal or problematic. If subfertility does occur and results solely from anovulation, then ovulation induction results in a close to normal fertility rate. An important concern is the associated metabolic abnormalities that may occur with the increased risk of the presence of insulin resistance. If a patient has the pattern of metabolic syndrome, all first-degree relatives, including males, are also of increased risk and should have similar screening performed.

Clinical diagnosis of PCOS is recommended. This diagnosis consists of chronic estrogenized anovulation with hyperandrogenism and exclusion of other causes of endocrine diseases. There are many evidences that linked interleukin serum levels and PCOS phenotype, such as IS-1b, which is enrolled in cell proliferation, differentiation, and apoptosis. Also; IL-6 is considered as a stabilizer of CRP that produced in the liver. Elevated levels of IL-18 are existed without relevance to insulin resistance and obesity, while IL-10 had no significant association with PCOS.

OBJECTIVE:
Evaluate the linkage (if any) between different metabolic, hormonal, and inflammatory markers in patients with PCOS.

PATIENTS AND METHODS:
A case-control study was conducted at the Department of Obstetrics and Gynecology / Baghdad teaching hospital-medical city, from January 2016 to January 2017. Thirty cases with Polycystic Ovary syndrome were enrolled in this study. Diagnosis of patients was made on the basis of clinical and biochemical findings and on bases of Rotterdam criteria (PCOS to be present if any 2out of 3 criteria are met): Oligo ovulation and/or anovulation, Clinical or and biochemical signs of hyperandrogenism, and polycystic ovaries (by gynecologic ultrasound). Exclusion criteria were Cushing’s syndrome, thyroid dysfunction, androgen secreting tumors, patients on hormonal contraceptives, acute infection, congenital suprarenal hyperplasia, hyperprolactinemia, and diabetes. A control group of 30 women without PCO or other diseases, with regular menstruation and matched for age and body mass index (BMI) were included. Data were collected according to the study protocol through full history and clinical examination and interviewing the study participants, using a standard pre-constructed data collection form (case sheet) for each patient which consisted of patient’s name and identification number. Demographic characteristics of the patient, including age, residence, family history, social and behavioral habits. History of the present illness. Medical and surgical history. Women's reproductive health history. The Body mass index (BMI) then calculated and expressed as weight in kg divided by height in meters squared (kg/m²).

Venous blood samples were obtained in early follicular phase of menstrual cycle [day 2-6] in eumenorhoeic, oligomenorhoeic women or at random in amenorhoeic ones for LH, FSH and testosterone. Serum level of Human Interleukin 1β(IL-1β) was quantitatively determined by means of sandwich ELISA. Fasting blood samples were also tested for the levels of cholesterol, and triglycerides. CRP measure method was based on the classical detection of antigen-antibody interaction through precipitation and agglutination reactions. Study protocol was approved by obstetrics and gynecology department, college of medicine, university of Baghdad. Verbal consent was required for all involved patients.

Statistical analysis:
Data of the study participants (30 PCOS women) and the 30 healthy women were analyzed by the statistical package for social sciences (SPSS) software for windows, version 20, 2011. Descriptive statistics were presented as mean ± SD, frequencies (number of patients) and proportions (%). Chi square test was used to compare frequencies and the students’ t test was used to compare two means, ANOVA test was used to
compare more than 2 means. Statistical significance was touched when p ≤ 0.05.

RESULTS:
There were 30 women (50%) with PCOS (cases group) and another 30 (50%) healthy women as (control group) were enrolled in this case control study.

Clinical and laboratory data
As it is shown in table 1, clinical and laboratory data revealed that the mean age of the PCOS cases was (24.9 ± 6.3) years and for control it was (25.3 ± 5.8) years.
The mean BMI was (27.8 ± 4.2) ranging between (18- 37.3) kg/m² in PCOS group and it was 26.4 ±0.7 ranging between (19.4-36.8) kg/m² in controls. In PCOS women and controls the mean triglyceride levels (mg/dl) were 124.8 ± 11.3 and 128.7 ±13.2 respectively. No statistically significant differences had been found in between both study groups regarding the age, BMI and triglycerides, where P-value > 0.05. The mean cholesterol levels were significantly higher in PCOS group rather than controls; (188.3 ± 7.2) vs. (168.7 ± 6.1) respectively, (P=0.001). LH and FSH levels were significantly different in between study groups; mean LH level was higher in PCOS women than control (13.3 ± 2.4 IU/L) vs. (5.85 ± 1.47) IU/L, (P=0.003), but looking into mean FSH level, it was lower in PCOS cases than in controls; (3.62 ± 1.22) vs. (6.4 ± 1.35) IU/L respectively, with (P=0.002).
The mean serum Testosterone in PCOS group was (1.76 ± 0.31) ng/ml which was significantly higher than (0.52 ± 0.17) ng/ml of controls, P<0.001. Although, IL1-B was detectable in two PCOS cases (6.67%) and only one control case (3.34%), the difference was statistically not significant.
C-reactive protein was positive in 13 PCOS women (36.7%) and two (6%) controls, so that; the difference was statistically significant.

Table 1: Clinical and laboratory data of PCOS and healthy control women.

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Women with PCOS No. = 30</th>
<th>Control women No. = 30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24.9 ± 6.3 (17 – 35)</td>
<td>25.3± 5.8 (18 – 35)</td>
<td>0.042</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 4.2 (18- 37.3)</td>
<td>26.4 ±0.7 (19.4-36.8)</td>
<td>0.056</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>124.8 ± 11.3(79.6-165.2)</td>
<td>128.7 ±13.2 (71.4 – 162.3)</td>
<td>0.069</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>188.3 ±7.2 (160.2-224.5)</td>
<td>168.7 ±6.1 (148.7 – 189.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>LH(IU/L)</td>
<td>13.3 ± 2.4 (8.2 – 22.9)</td>
<td>5.85 ± 1.47 (3 – 9)</td>
<td>0.003</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>3.62 ± 1.22 (1.5 – 6)</td>
<td>6.4 ± 1.35 (5 – 9)</td>
<td>0.002</td>
</tr>
<tr>
<td>S. Testosterone (ng/ml)</td>
<td>1.76 ± 0.31 (0.68 – 3.4)</td>
<td>0.52 ± 0.17 (0.3 – 0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-1B detectable</td>
<td>2 (6.67%)</td>
<td>1 (3.34%)</td>
<td>0.682</td>
</tr>
<tr>
<td>Positive CRP n (%)</td>
<td>13 (36.7%)</td>
<td>2 (6%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* values are mean ± standard deviation (Range), unless mentioned. p-value was considered significant when it is ≤ 0.05.

BMI distribution:
BMI was categorized into 3 categories according to its measurement.
Accordingly; normal BMI is < 25 kg/m², while overweight is 25 – 29.9 kg/m², and obese is ≥ 30 kg/m². When these categories were put into the comparison between both study groups (PCOS and control), it had been found that 14 PCOS women...
(46.7%) and 17 controls (56.7%) had normal BMI (<25 kg/m\(^2\)), overweight (25 – 29.9 kg/m\(^2\)) was found in 9 PCOS women (30%) and 8 controls (26.7%), while 7 PCOS women (23.3%) and 5 controls (16.6%) were obese (≥ 30 kg/m\(^2\)). However; the difference between both groups was statistically not significant (P=0.81). All these findings are clear in figure 1 below.

**Figure 1: Comparison in between PCOS and control groups according to BMI categories**

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PCOS group: p-value = 0.012, while R = 0.54
Control group: p-value = 0.028, while R = 0.35

**Figure 2: Correlation between BMI and triglycerides.**
Correlation between BMI and other parameters:

**BMI and Triglycerides**

In figure 2, the correlation between BMI and triglycerides revealed that obese subjects in both study groups had the higher level of triglycerides and there was a positive (direct) correlation between BMI and triglyceride level. From other point of view; the correlation was stronger and more significant in PCOS group than in controls (R=0.54, P=0.012) vs. (R= 0.35, P=0.028), respectively.

![Correlation between BMI and cholesterol level.](image)

**PCOS group:** p-value = 0.002, while R = 0.62  
**Control group:** p-value = 0.004, while R = 0.48

**Figure 3: Correlation between BMI and cholesterol level.**

**BMI and Cholesterol:**
The trend of correlation between BMI and cholesterol was not much different from that with triglycerides.

It had been significantly found that cholesterol level was directly correlated with BMI, on the other hand, the PCOS had a clear effect on the trend of this correlation where the correlation was stronger and more significant in PCOS group than controls, as illustrated in figure 3, where (R=0.62, P=0.002) vs. (R= 0.42, P=0.004), respectively.

**BMI and LH**
The mean LH level of PCOS women was much higher than that of controls across all categories of BMI.

The much difference was among obese women. Correlation between BMI and LH was significantly positive correlation and it was more obvious in PCOS group.

For more details please go to figure 4.
PCOS group: p-value = 0.001, while R = 0.72
Control group: p-value = 0.003, while R = 0.61

Figure 4: Correlation between BMI and LH level.

BMI and FSH
Looking into table 2; FSH showed positive moderate correlation with BMI in both studied groups. However; the correlation was more significant and stronger in controls rather than PCOS women, (R=0.32, P=0.037) vs. (R=0.47, P=0.026).

Table 2: Correlation between BMI and FSH level.

<table>
<thead>
<tr>
<th>BMI categories</th>
<th>PCOS Mean FSH (n=30)</th>
<th>Control Mean FSH (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal &lt; 25 kg/m²</td>
<td>2.21 ± 0.93 m IU/L</td>
<td>5.22 ± 1.20 m IU/L</td>
</tr>
<tr>
<td>Overweight 25 – 29.9 kg/m²</td>
<td>3.32 ± 1.24 m IU/L</td>
<td>6.30 ± 1.28 m IU/L</td>
</tr>
<tr>
<td>Obese ≥ 30</td>
<td>5.68 ± 1.32 m IU/L</td>
<td>7.11 ± 1.42 m IU/L</td>
</tr>
</tbody>
</table>

BMI and Serum Testosterone:
Serum testosterone was found to have weak correlation with BMI in both PCOS and controls group (R=0.27, P=0.041) and (R=0.19, P=0.033) respectively. This was different when the correlation assessed in relation to the total BMI and not in categories (as shown previously in table 1). So that; the correlation still significant across BMI categories as explained in table 3.
Table 3: Correlation between BMI and Testosterone level.

<table>
<thead>
<tr>
<th>BMI categories</th>
<th>PCOS Mean testosterone level (n=30)</th>
<th>Control Mean testosterone level (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal &lt; 25 kg/m²</td>
<td>1.34 ± 0.11 m IU/L</td>
<td>0.37 ± 0.10 m IU/L</td>
</tr>
<tr>
<td>Overweight 25 – 29.9 kg/m²</td>
<td>1.68 ± 0.32 m IU/L</td>
<td>0.43 ± 0.2 m IU/L</td>
</tr>
<tr>
<td>Obese ≥ 30</td>
<td>2.49 ± 0.62 m IU/L</td>
<td>0.71 ± 0.36 m IU/L</td>
</tr>
<tr>
<td>R</td>
<td>0.27 m IU/L</td>
<td>0.19 m IU/L</td>
</tr>
<tr>
<td>P-value</td>
<td>0.041</td>
<td>0.033</td>
</tr>
</tbody>
</table>

BMI and IL-1B:
Table 4 shows the correlation between the BMI and IL-1B, where it had been found that the IL-1B was detectable in only 2 PCOS women (6.7%) with one control (3.4%), and it was not detectable in the remaining 28 PCOS women and 29 controls, further distribution according to BMI categories, revealed that one of the 2 PCOS cases had normal BMI value and the other was overweight, and the only one control with detectable IL-1B had a high BMI (overweight). By using Fisher’s exact test (alternatively used when chi square can’t be applied due small values), there was no statistically significant correlation found between BMI and IL-1B, P > 0.05. Furthermore, the mean BMI of the 2 PCOS women with detectable IL-1B was (25.2 ± 0.71) kg/m² and of remaining 28 PCOS without detectable IL-1B was (25.7 ± 5.6) kg/m² with no significant difference, P > 0.05. The only one control women with detectable IL-1B had a BMI value of 28.3 kg/m² which was within the overweight reference value, and the mean BMI of the remaining 29 controls was (25.8 ± 5.7) kg/m². However; no statistically significant differences had been found in mean BMI between those with and without detectable IL-1B, in both study groups, P > 0.05.

Table 4: Correlation between BMI and IL-1B level of studied groups.

<table>
<thead>
<tr>
<th>BMI categories</th>
<th>PCOS group</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Normal &lt; 25 kg/m²</td>
<td>1 (6.7%)</td>
<td>14 (93.3%)</td>
</tr>
<tr>
<td>Overweight 25 – 29.9 kg/m²</td>
<td>1 (10%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Obese ≥ 30</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (6.7%)</td>
<td>28 (93.3%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test = 0.79 P=0.62 (not significant)

BMI and C-reactive protein (CRP)
The correlation between CRP and BMI revealed that out of the 13 CRP positive PCOS, 6 had normal weight, 4 were overweight and 3 were obese. While the only 2 CRP positive controls were of normal weight, and there was no statistically significant correlation between CRP and BMI (P>0.05).

DISCUSSION:
Many studies have suggested a correlation between the PCOS and metabolic abnormalities. However; it is not clear whether the risk for the development of alterations in the metabolism is related to the...
endocrine alterations observed in PCOS patients such as hyperandrogenemia or whether the metabolic abnormalities are a consequence of the metabolic and anthropometric abnormalities observed in some of these patients \(^9, ^{10}\). In the current case control study, no statistically significant differences between both groups (PCOS and control cases) had been found with regard to age, BMI, and triglycerides levels, while it had been significantly found when women with PCOS had higher cholesterol levels than controls. This is consistent with other studies reporting many patients with polycystic ovary syndrome (PCOS) also had features of the metabolic syndrome, including insulin resistance, obesity, and dyslipidemia compared to weight matched controls. \(^{11}\)

Also, these findings of our study agreed with the results found by a study done in Oman. \(^{12}\) Regarding the hormonal assay measurements; the mean LH level of PCOS women was much higher than that of controls across all categories of BMI, and a positive correlation had been found between BMI and LH level. This correlation was more obvious in PCOS group than controls. FSH showed positive moderate correlation with BMI in both studied group, but it was more significant and stronger in controls rather than PCOS women. These findings indicated that LH and FSH could be a useful predictor for PCOS in women with different BMI categories, which were in line with that published in literatures and other studies. \(^{3,13,14}\)

It is well known that women with PCOS tend to have higher mean LH concentrations but normal or low levels of follicle stimulating hormone FSH with a corresponding increase in the LH:FSH ratio on serum testing in 50-70% of women. \(^{15}\)

Unfortunately, a lack of sensitivity and specificity precludes the use of LH as a diagnostic tool. \(^{16, 17}\)

The current study reported a weak correlation between serum testosterone and BMI within both PCOS and control groups (R=0.27, P=0.041) and (R=0.19, P=0.033) respectively. These were disagreed with the results that reported by an author from United States who said that androgen production and metabolism in women was altered with obesity resulting in higher serum levels with increasing BMI and waist circumference. \(^{18}\)

Furthermore, there were some data from Poland found a significant linear positive correlation. \(^{19}\)

Cytokines are associated with chronic inflammatory diseases (CIDs), IL-1B appears to be the primary mediator of inflammation in CID. \(^{11, 19}\)

In the present study, IL-1B detected in two PCOS women and only one control with no statistically significant correlation between BMI and IL-1B detection. This finding was in harmony with the Austrian paper, in which authors did not ascertain any significant association between presence of the IL-1B within PCOS women and controls. \(^{20}\)

A statistically significant difference had been found in our data between cases and controls regarding the presence of C-reactive protein (CRP), which was also mentioned by more than one researcher. \(^{21, 22}\)

These findings were confirmed by a large number of diverse studies evaluating various populations of women with PCOS, such as a recent meta-analysis which evaluated 31 clinical trials and included 2359 women with PCOS and 1289 controls, concluded that CRP in women with PCOS was greater than in healthy subjects when adjusted for BMI. \(^{23}\)

Our findings indicated a significant association between PCOS and presence of inflammatory markers when the effect of BMI was controlled.

CONCLUSION:

PCOS was clearly associated with an increased incidence in inflammatory markers (especially CRP), and metabolic factors (such as serum cholesterol, testosterone, and LH), which were mainly due to PCOS rather than BMI.

REFERENCES:


INFLAMMATORY MARKERS IN POLYCYSTIC OVARY

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