THE ROLE OF INFLAMMATION AND OXIDATIVE STRESS IN THE PATHOGENESIS OF POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder of unknown etiology characterized by anovulation and hyperandrogenemia associated with other symptoms mainly insulin resistance. Methods: 60 clinically diagnosed PCOS patients according to Rotterdam criteria and 30 apparently healthy subjects have been included in this case control study. Results: There were significant increases in inflammatory markers including hs-CRP (P=0.001), total WBC count (P=0.03) and Erythrocyte sedimentation rate (P=0.001) in PCOS group compared to control subjects. Furthermore, serum malondialdehyde (MDA) levels were significantly higher in PCOS patients (P=0.005). Conclusion: There is existence of low grade chronic inflammation as well as oxidative stress represented by malondialdehyde that play a pivotal role in PCOS pathogenesis.

KEYBOARD: Polycystic ovary syndrome, malondialdehyde and hyperandrogenemia.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder affecting around 8-10% of women in their reproductive age characterized mainly by hyperandrogenism and chronic anovulation associated with other variable manifestations such as insulin resistance, dyslipidemia and hirsutism. Even though the etiology of PCOS is not well understood, genetic analysis showed that polymorphisms in certain genes developing insulin resistance and hyperinsulinemia seems to have a notable influence on hypothalamo-pituitary-ovarian
axis. Moreover, this results in excessive ovarian androgen synthesis and consequently altering LH/FSH ratio and eventual outcome is anovulation. Nowadays, the clinical diagnosis of PCOS is done based on Rotterdam criteria (PCOM; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Accordingly, the accurate diagnosis may be achieved if at least two out of three of the following criteria are met; hyperandrogenism; oligomenorrhea or amenorrhea or anovulation and polycystic ovary represented by multiple immature ovarian follicles at the day of ovulation.

Although the majority of previous studies focused on the clinical, biochemical, metabolic and cardiovascular aspects of PCOS as well as their risk factors, recent studies have been studying the contribution of inflammation and oxidative stress to the pathogenesis of PCOS and the mechanisms underlying their direct influence on anovulation. Despite the role of oxidative stress is well documented in these studies, the exact evaluation of inflammatory mediators is still unknown. Generally, studies have shown conflicting results, some of them emphasized the significant elevation in inflammatory markers whereas others did not represent clear conclusions. In this study, we aim to evaluate the status of both inflammation and oxidative stress in the pathogenesis of PCOS with a particular focus on the possible mechanisms underlying their contribution to infertility.

SUBJECTS AND METHODS

This case control study was conducted at Department of Physiology, School of Medicine, Faculty of Medical Sciences, University of Duhok, Kurdistan Region of Iraq from January 2015 to May 2015.

Two groups of subjects were included in this study, the first group composed of 60 patients who had been clinically diagnosed as polycystic ovary syndrome (PCOS) according to Rotterdam criteria (oligo- and/or anovulation or clinical and/or biochemical hyperandrogenism or polycystic ovaries on ultrasound). After receiving an approval letter from research ethical committee, form C was given to all participants for obtaining their written consent. The second study group comprised of 30 apparently healthy individuals as control. To confirm the control subjects are free of oxidative stress and inflammatory conditions, necessary laboratory and clinical investigations were carried out. Questionnaires were completed for all subjects include gender, age, blood pressure, hirsutism, body mass index (BMI) and PCOS duration.
Five ml of blood was collected from each subject and divided into two parts; the first part collected in EDTA tube and used for hematological assessments whereas the rest of the sample placed into a plain tube and centrifuged. The serum samples were collected into epindorff tube followed by labeling and eventually frozen under – 28 °C to be used for biochemical examinations later. Serum levels of high sensitive C-reactive protein were measured by ELISA (Monobind Inc., USA Kit), erythrocyte sedimentation rate (ESR) by Westergreen method in addition to manual total WBC count were used as markers of inflammation. On the other hand, Malondialdehyde (MDA, nmol/ml) which is the end product of lipid peroxidation was used for quantitative measurement of oxidative stress in serum samples using thiobarbituric acid method. Hormonal assays including follicular stimulating hormone, luteinizing hormone and prolactin were performed at the second day of menstrual cycle using enzyme immunoassay technique (Biomerieux manufacturer, France).

Statistical analysis was done using SPSS version 18 (Chicago, USA). All variables were described by mean and standard error (SE) and the independent t test used to determine the statistical significance of difference in mean between two groups. P values of 0.05 or less were considered statistically significant whereas the values of 0.005 or less were considered highly significant.

RESULTS

Based on body mass index (BMI), PCOS patients had a highly statistically significant BMI (mean 27.03) compared to control subjects (P= 0.004).

Table 1: Basic parameters of PCOS patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index (BMI)</td>
<td>27.03 ± 3.24</td>
<td>23.9 ± 2.06</td>
<td>0.004</td>
</tr>
<tr>
<td>% of Positive hirsutism</td>
<td>80</td>
<td>12</td>
<td>0.041</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.34 ± 2.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.1 ± 4.71</td>
<td>29.7 ± 6.88</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Among PCOS group, the percentage of patients who had positive hirsutism was %80 and was significantly higher compared to controls (P= 0.041) (Table 1).

Table 2: Comparison in blood pressure between study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS patients</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systollic Blood Pressure</td>
<td>120 ± 9.09</td>
<td>100 ± 6.67</td>
<td>0.035</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>80 ± 4.91</td>
<td>75 ± 5.67</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Regarding arterial blood pressure measurement, results showed a significant increase in systolic blood pressure (P= 0.03) in PCOS group compared to control whereas the diastolic blood pressure did not show a significant difference (Table 2).

**Table 3: Hormonal profile among study groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS patients</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>5.43 ± 2.33</td>
<td>7.57 ± 1.53</td>
<td>0.007</td>
</tr>
<tr>
<td>LH</td>
<td>9.72 ± 2.18</td>
<td>4.52 ± 1.82</td>
<td>0.14</td>
</tr>
<tr>
<td>Prolactin</td>
<td>32.3 ± 2.52</td>
<td>13.63 ± 1.29</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Hormonal profile of PCOS subjects showed a statistically significant decrease in follicular stimulating hormone (FSH) levels compared to controls (P < 0.05) whereas the patient's luteinizing hormone (LH) showed an obvious increase although it was not significant (P=0.14). Moreover, a significant hyperprolactinemia was observed in patients (P <0.05) rather than controls (Table 3).

**Table 4: Inflammatory markers among study groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS patients</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC Count (Cells / mm³)</td>
<td>8800 ± 2.66</td>
<td>7389 ± 1.28</td>
<td>0.001</td>
</tr>
<tr>
<td>ESR (mm/1st hour)</td>
<td>15.6 ± 7.71</td>
<td>6.75 ± 4.31</td>
<td>0.03</td>
</tr>
<tr>
<td>hs-CRP (mg / dL)</td>
<td>4.05 ± 2.81</td>
<td>1.5 ± 4.59</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Surprisingly, all inflammatory markers including total WBC count, erythrocyte sedimentation rate (ESR) and high sensitive C-reactive protein (hs-CRP) showed a significant increase in PCOS patients compared to control group represented by (P=0.01), (P=0.03) and (P=0.01) respectively (Table 4).

**Table 5: Malondialdehyde assay**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS patients</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (nmol / ml)</td>
<td>2.08 ± 72.3</td>
<td>0.76 ± 4.07</td>
<td>0.005</td>
</tr>
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</table>

Interestingly, serum levels of malondialdehyde (MDA) which is the end product of lipid peroxidation and regarded as a marker of oxidative stress assessment revealed a highly statistically elevation in PCOS (P= 0.005) compared to control subjects (Table 5).

**DISCUSSION**

To display the association between BMI and hyperandrogenemia in this study, BMI (P=0.004) as well as the percentage of positive hirsute were significantly higher in PCOS group. This is indicated in previous literature that has shown a positive correlation between
obesity and excess androgen levels and has been regarded as key players in orchestrating PCOS pathogenesis.\[13\] In addition, our study have paid attention to the arterial blood pressure as one of the cardiovascular risk factors in PCOS which showed a significant increase in systolic blood pressure in PCOS patients and this is clearly stated in earlier studies.\[14\] To further confirm the hormonal profile of PCOS patients, our results showed a significant decrease in FSH and a non significant but remarkable elevation in LH in PCOS patients which indicates altered LH/FSH ratio. This is consistent with other studies concluding that altered LH/FSH ratio prevents follicular maturation during follicular phase.\[15\]

Despite the involvement of oxidative stress in initiating and progressing PCOS pathogenesis is well documented, the association between inflammation and PCOS is still unclear. Surprisingly, results of the present study showed a significant increase in all inflammatory parameters including high sensitive hs-CRP, total WBC count and erythrocyte sedimentation rate (ESR) among study group compared to healthy subjects regardless of their normal ranges. This indicates persistence of low grade chronic inflammation exerting a direct influence on anovulation. These results are consistent with previous literature concluding that low grade inflammation is demonstrated by moderately elevated levels of hs-CRP concentrations.\[16, 17\] The possible mechanism underlying the relevancy of elevated CRP levels to PCOS pathogenesis may be due to its crucial role in endothelial dysfunction and complement activation in addition of releasing chemo-attractants such as intracellular adhesion molecules (ICAM) resulting in to recruitment of innate immune cells particularly macrophages.\[18, 19\]

Other studies stated that obesity and insulin resistance seem to play a key role in initiation of the inflammatory immune response because they result in accumulation of free fatty acids (lipotoxicity) which leads to nuclear factor kB (NF kB) activation and subsequently release of inflammatory cytokines including IL-6 and TNFα.\[20\] Moreover, other studies emphasized our results regarding elevated total leukocyte count and concluded that WBC count is slightly elevated in PCOS patients who had insulin resistance although they were within the normal range.\[21\]

Malodialdehyde which is the end product of lipid peroxidation also showed a significant elevation in PCOS patients compared with women with normal ovulation. This is consistent with previous studies concluding that there is a remarkable elevation in various oxidative
stress markers including lipid peroxidation (malondialdehyde) in PCOS patients who have insulin resistance and high androgen levels. Studies concluded that reactive oxygen species are produced in response to hyperglycemia and hypertriglyceridemia. The proposed impact of oxidative stress particularly reactive oxygen species (ROS) on ovarian functions may be due to its direct influence on ovulation through decreasing granulosa cells luteinization and oocyte maturation which eventually leads to anovulation.

In conclusion, we have demonstrated for the first time the impact of inflammatory mediators along with the oxidative stress on ovarian functions in women having PCOS. This indicates existence of low grade chronic inflammation represented by mild elevation of inflammatory mediators particularly CRP accompanied with significantly elevated malondialdehyde that play a critical role in ovarian dysfunction that eventually cause anovulation. For that reason, targeting inflammation and oxidative stress could improve ovarian dysfunctions and subsequently improving or restoring normal ovulation to a great extent.

REFERENCES
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