Evaluation Of Oxidative Stress And Its Association With Pro-Inflammatory Cytokines In Obese, And Non-Obese PCOS Subjects.

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Abstract

Introduction: Polycystic ovarian syndrome (PCOS) is a common endocrinological disorder of reproductive age female. The etiology of PCOS is not completely understood. Several publications nowadays demonstrate the relationship between chronic low-grade inflammation and mitochondrial dysfunctions induced by oxidative stress.

Methods: This hospital based cross-sectional study, was conducted among reproductive age females. The study includes 73 obese PCOS, 55 Lean PCOS subjects and 15 non-obese PCOS subjects on the basis on transvaginal sonography and BMI. The anthropometric parameters were: BMI, Waist Hip Ratio (WHR), systolic and diastolic blood pressure by sphygmomanometer. Serum samples were collected to measure: oxidative stress (MDA), lipid profile, and pro-inflammatory cytokines (HsCRP, IL-6, IL-18 & TNF-α).

Results: The BMI and MDA levels were significant in obese-PCOS and lean PCOS group (p <0.001). Whereas, in the obese-PCOS and non-PCOS obese group MDA, triglycerides, HDL, VLDL, IL-6, IL-18, and TNF-α were significant respectively.

Conclusion: It concluded from this study that obesity may contribute to dyslipidemia and elevated oxidative stress levels in reproductive age females irrespective of PCOS. Therefore, obesity in addition to known features such as dyslipidemia, hypertension and disturbed oxidative stress response, may be contributing factors to increase the risk of future cardiovascular diseases in obese women.

Keywords: Obesity, Oxidative stress, Polycystic ovarian syndrome, pro-inflammatory cytokine.

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrinological disorders of females of reproductive age with a prevalence rate of about 5-14% (1). The etiology of PCOS is not completely understood. Several publications nowadays demonstrate the relationship between chronic low-grade inflammation and mitochondrial dysfunctions induced by oxidative stress (2,3). The evidence suggests that the women suffering from PCOS have increased levels of lymphocyte and monocyte counts, as well as elevated levels of pro-oxidants like C-reactive protein (CRP), pro-inflammatory cytokine (TNF-α), interleukins (IL-6, IL-18) and elevated levels of lipid peroxidation in comparison with the healthy controls (4,5). It is known that the increased production of reactive oxygen species (ROS) initiates the activation of inflammatory responses in the PCOS subjects because of the mitochondrial damage and dysfunctioning, which play a vital role in the development of oxidative stress and leads to vicious cycle resulting in activation of the inflammatory response (6).

Oxidative stress is defined as the imbalance between the production of free radicals which increase the chemical reactivity and antioxidant defenses buffering the oxidative damage (7). Several studies suggest that oxidative stress is significantly increased in women with PCOS in comparison with healthy controls and is significantly correlated with
obesity, insulin resistance, cardiovascular diseases, hyper-androgenemia, and chronic inflammation (9). This may be due to a response from mononuclear cells (MNC) of women with PCOS as macrophages derived from MNC are the primary source of pro-inflammatory markers. These pro-inflammatory and anti-inflammatory markers are produced and released excessively in adipose tissues and abdominal adiposity/obesity may contribute to increased inflammation as pro-inflammatory markers as excess (9). However, there is no concrete relationship between the pro-inflammatory markers and oxidative stress in relation to abdominal obesity is defined to form a clear correlation between PCOS-generated weight status in obese PCOS, lean PCOS subjects, and non-PCOS obese subjects.

Material & methods

144 females of the reproductive age group were included in the present cross-sectional study. Who visited the obstetrics & gynecology department of Santosh medical college and hospital, Ghaziabad. The PCOS cases were confirmed by Transvaginal sonography (TVS), and divided into three groups:

- **Group I:** 73 were obese PCOS
- **Group II:** 56 were lean PCOS subjects
- **Group III:** 15 non-PCOS obese

**Exclusion criteria:** Women with diabetes, pregnant women, women on oral contraceptive pills, and post-menopausal women with PCOS were excluded from the study.

A general clinical examination was performed for all the participants. Anthropometric data (age, height, weight, BMI, waist and hip ratio). BMI was calculated and it was considered normal if the values were less than 25, overweight for the values between 25 to 29.9 if the values were greater than 29.9 were considered obese.

Ethical clearance was obtained from Institutional Ethical Committee. Subjects were informed and written consent was taken from each participant.

**Methodology:**

**Anthropometric parameters:** the anthropometric factors like BMI were calculated by

\[
\text{BMI} = \frac{\text{Weight (in Kg)}}{\text{Height (m)}} \times 10000
\]

The systolic and diastolic blood pressure was measured by a sphygmomanometer.

**Biochemical parameters:**

100μl serum and 0.50μl of EDTA anticoagulated plasma were separated and stored at -20°C in Eppendorf until the tests were performed. The concentration of MDA, in venous blood was determined by Kei Satoh’s method, IL-6 was determined by the ECLIA method, and IL-18 and TNF-α were determined by the ELISA method. The lipid profile was estimated for total cholesterol, triglycerides (TG), low-density lipoproteins (LDL), high-density lipoprotein, and very low-density lipoproteins (VLDL) by Roche/Hitachi Cobas C systems.

**Statistical analysis**

Descriptive statistical methods such as mean and standard deviation were applied to summarize continuous variables. The normality of the variables was tested by using the Shapiro-Wilk test and Kolmogorov Smirnov test, which show that the data was not normally distributed. The non-parametric Mann-Whitney U-test was performed to identify whether both groups come from a statistically different population. However, results were shown in the form of mean ± SD. Further, Person’s correlation coefficient was used to calculate the bivariate correlation between study variables. The data were analyzed using IBM SPSS 26 trial version.

**Results and Discussion**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obese PCOS (n=73)</th>
<th>Lean PCOS (n=55)</th>
<th>Non-PCOS Obese (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26.74 ± 5.897</td>
<td>25.85 ± 5.101</td>
<td>25.73 ± 4.07</td>
</tr>
<tr>
<td>BMI</td>
<td>36.18 ± 4.87</td>
<td>21.63 ± 2.52*</td>
<td>34.79 ± 4.02</td>
</tr>
</tbody>
</table>
Table 1. Comparison of clinical, and biochemical parameters among obese PCOS, lean PCOS, and non-PCOS obese subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese PCOS</th>
<th>Lean PCOS</th>
<th>Non-PCOS</th>
<th>*</th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHR</td>
<td>0.88 ± 0.15</td>
<td>0.90 ± 0.16</td>
<td>0.90 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>120.05 ± 12.91</td>
<td>117.51 ± 12.09</td>
<td>114.47 ± 11.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>83.34 ± 8.05</td>
<td>82.31 ± 8.13</td>
<td>84.01 ± 11.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>4.96 ± 1.52</td>
<td>4.39 ± 1.69*</td>
<td>3.48 ± 1.35*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>180.72 ± 25.96</td>
<td>185.81 ± 34.42</td>
<td>181.71 ± 24.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>122.85 ± 45.02</td>
<td>107.80 ± 30.98</td>
<td>83.45 ± 14.63*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>31.71 ± 11.99</td>
<td>32.29 ± 11.81</td>
<td>42.27 ± 7.26*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>124.44 ± 30.07</td>
<td>131.95 ± 37.64</td>
<td>122.74 ± 26.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>24.57 ± 9.0</td>
<td>21.56 ± 6.19</td>
<td>16.69 ± 2.92*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>0.99 ± 0.45</td>
<td>0.86 ± 0.54</td>
<td>0.82 ± 0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>11.64 ± 2.38</td>
<td>12.02 ± 3.75</td>
<td>4.68 ± 3.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.02 ± 0.40</td>
<td>1.09 ± 0.41</td>
<td>0.59 ± 0.12*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>1.01 ± 0.35</td>
<td>0.94 ± 0.36</td>
<td>0.44 ± 0.14*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (*) significant at the 0.05 level using Mann-Whitney U Test

The mean and standard deviation of anthropometric and biochemical parameters of the study population are shown in table 1. The mean age was 26.74 ± 5.897 for obese PCOS subjects, 25.85 ± 5.101 for lean PCOS subjects, and 25.73 ± 4.07 for non-PCOS obese subjects. No statistically significant change was observed between all three groups concerning age.

The Waist Hip Ratio was high in obese-PCOS subjects in comparison with lean-PCOS subjects and non-PCOS obese subjects. However, non-significant which is in accordance with the earlier studies; suggesting that the women with PCOS are more prone to upper body obesity, i.e. independent of BMI measured by increased waist circumference and waist-hip ratio, when compared with non-PCOS BMI, matched subjects (10). This may be due to high rates of glucose intolerance leading to dysfunctioning of insulin and β-cells in PCOS, and upper body obesity plays an important role to exacerbates the defect (10). Therefore, the reproductive age females with upper body obesity and PCOS are more prone to glucose intolerance which further leads to central or abdominal obesity.

The systolic blood pressure was high in obese-PCOS subjects in comparison with lean-PCOS subjects and non-PCOS obese subjects but not significant. Whereas, the diastolic blood pressure was high in non-PCOS obese subjects in comparison with obese-PCOS subjects but not significant. The pathophysiology of hypertension in PCOS is not completely understood, but several mechanisms are responsible for the development of hypertension in PCOS such as hyperandrogenism, insulin resistance, obesity, and increased sympathetic nervous system activity (11).

Some studies suggest that hyperandrogenism is associated with systolic and diastolic blood pressure in PCOS subjects irrespective of obesity and insulin resistance (12). The MDA level was high in obese-PCOS subjects in comparison with lean-PCOS subjects and non-PCOS obese subjects and significant. This supports the previous studies which suggest that obesity elevates systemic oxidative stress, and elevated levels were observed when obesity is associated with abdominal or central obesity (12).

Moreover, elevated MDA levels are also associated with dyslipidemia and insulin resistance (13). In the present study, we found that the total cholesterol level was high in lean PCOS and non-PCOS subjects in comparison with obese-PCOS subjects but not significant in both the groups. Whereas, the serum LDL cholesterol level was high in lean PCOS and obese-PCOS subjects in comparison with non-PCOS obese subjects but there was no significant difference between the groups. However, the triglyceride level and VLDL cholesterol were significantly high in obese-PCOS subjects in comparison with non-PCOS obese subjects. However, HDL cholesterol level was
significantly high in non-PCOS subjects in comparison with obese-PCOS subjects. This shows that obesity alone does not alter the triglyceride, HDL cholesterol, and VLDL levels significantly as compared with the rest of the lipid profile.

Furthermore, in the present study, we found that the HsCRP level was non-significantly high in obese-PCOS subjects in comparison with lean-PCOS subjects and non-PCOS obese subjects. However, in contradiction to earlier studies that show elevated CRP levels in PCOS subjects not because of obesity but due to polycystic ovaries themselves (14,15).

The levels of pro-inflammatory markers i.e. IL-6, IL-18, and TNF-α were found to be significantly elevated in obese-PCOS and non-PCOS obese groups whereas in the obese-PCOS in lean-PCOS subjects these markers were elevated but the increase was not significant. In agreement with previous studies that demonstrate that elevated levels of pro-inflammatory markers in PCOS subjects are associated with central fat excess (16). The elevated inflammatory markers along with genetic markers were shown to be higher in PCOS patients and hs-CRP, IL-6, IL-18, and TNF-α were found to correlate in the PCOS subjects with BMI-matched controls (17-19).

Conclusion
Polycystic ovarian syndrome is the most common endocrinological disorder in females of reproductive age. In our study, it is seen that the MDA levels were significantly increased in both groups. The parameters of lipid profile were significantly high in the obese-PCOS and non-PCOS groups. So, we conclude that obesity may contribute to dyslipidemia and elevated oxidative stress levels in reproductive age females irrespective of PCOS. Therefore, obesity in addition to known features such as dyslipidemia, hypertension and disturbed oxidative stress response, may be contributing factors to increase the risk of future cardiovascular diseases in obese women. Besides the disturbed oxidative stress response obese-PCOS and non-PCOS obese subjects shows significantly elevated levels of pro-inflammatory cytokines (IL-6, IL-18, and TNF-α) levels also because obesity may contribute to increased inflammation in the body. Hence we can conclude that the obese females of reproductive age irrespective of PCOS are more prone to develop oxidative stress and diseases associated with it.

Limitations
Our study has a few limitations. First, our sample size for non-PCOS obese subjects is relatively small and hence cannot be generalized. Second, to confirm the validity of the findings additional research on different populations is recommended. Due to the limited population, the conclusion must be drawn with caution. Large demographic studies on this subject are needed to conclude the findings.

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