Original article

Study of oxidative stress and antioxidant levels in polycystic ovarian disease

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Abstract:

Background: Polycystic ovary disease (PCOD) is a common but complex endocrine disorder and is a major cause of anovulation and consequent infertility. The stressful lifestyle is leading to increased prevalence of polycystic ovarian disease in young adolescent and early reproductive population and its association with many ongoing complications such as infertility, obesity, insulin resistance, dyslipidemia, endothelial dysfunction and overt diabetes mellitus. The study was undertaken to analyze the oxidative stress in females having polycystic ovarian disease and compare them with age and gender matched healthy subjects. We correlated oxidative stress by measuring serum Malonyldialdehyde-(MDA) levels and antioxidant levels by measuring serum Vitamin C levels in PCOD patients.

Study methodology: Oxidative stress were measured in 60 Subjects within the age group of 20 to 30 years were selected and divided into two groups. Group I consisted of 30 diagnosed female patients of PCOD and Group II comprised of 30 age matched control group with normal menstrual cycles. To assess oxidative stress, serum Malonyldialdehyde (MDA) was estimated by Buege and Aust method while antioxidant level was assessed by estimation of serum vitamin C by kyaw A method. Serum MDA level and serum vitamin C levels were compared by applying unpaired t test. Comparison between oxidative stress and antioxidant level were analyzed by applying Pearson’s coefficient.

Results: The oxidative stress was significantly increased and had significant correlation in PCOD patients compared with the healthy controls.

Conclusion: In the present study, polycystic ovarian disease patients was having oxidative stress which may leads to complication of disease like infertility as well as other systemic disorders e.g. diabetes mellitus, cardiovascular dysfunction, dislipidemia and endometrial carcinoma. Hence in the polycystic ovarian disease patients antioxidants can be of help to fight against these free radicals and prevent the future complications.

Key word: Polycystic ovarian disease, Oxidative stress, Malonyldialdehyde, Vitamin C.

Introduction:

Polycystic ovarian disease (PCOD) is a common health problem which is increasing in teenage girls and young women. It is one of the most common endocrine disorders of women in reproductive age group, with prevalence of 15% [1] which occurs in almost all races and nationality and is a leading cause of infertility. [2] In India, the prevalence of PCOD is from 2.2% to 26%.

[3] PCOD is an anovulatory cause of infertility affecting 6-10% of premenopausal women. [4] PCOD often presents as hyperandrogenism, hirsutism
and oligomenorrhea or amenorrhea. Metabolic, endocrinologic and cardiovascular disorders may also coexist in PCOD.

The imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, which produces the oxidative damage is known as oxidative stress (OS).[5] Reactive oxygen species can affect a variety of physiological functions in the reproductive tract. When ROS increase to pathological levels like in PCOD, they are capable of inflicting significant damage to cell structures. It can modulate cellular functions and can impair the intracellular milieu, resulting in diseased cells or endangered cell survival. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize these damages, thereby adapting itself to the above stressful situation. The antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. [6] Reproductive cells and tissues remain stable when ROS production and the scavenging antioxidants remain in balanced state. Levels of ROS are controlled and kept at physiological levels within the ovary by various antioxidant systems like vitamin C, which is known to have a protective effect within the follicle. Vitamin C deficiency has been reported to result in ovarian atrophy, extensive follicular atresia and premature resumption of meiosis. [7]

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Malonyldialdehyde (MDA) is a marker of lipid peroxidation and it increases in oxidative stress states,[8] while vitamin C and vitamin E are non-enzymatic antioxidants and helps in scavenging these free radical. Therefore the role of oxidative stress in conditions such as polycystic ovarian disease is becoming more important.

**Pathophysiology**

Rise in MDA levels could be due to increased generation of Reactive Oxygen Species (ROS), which led to excessive oxidative damage in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. The probable cause of increase production of ROS in PCOD is as follows:

PCOD is characterized by insulin resistance. Because of this insulin resistance glucose utilization by body tissue is decreased. It leads to hyperglycemia and further increasing insulin production. Thus a state of hyperglycemia and hyperinsulinemia develops. Hyperglycemia in turn leads to increased generation of ROS from mononuclear cells. These increased ROS causes damage to all body cells including mononuclear cells leading to increased production of inflammatory markers like TNF-α and NF-Kappa B from the damaged cells. TNF-α is known mediator of insulin resistance which further aggravates the state of hyperglycemia and hyperinsulinemia. NF-Kappa B is an inflammatory transcription factor which leads to increase in the inflammatory state. The mononuclear cells of women with PCOD are increased in this inflammatory state. Persistent hyperglycemia in PCOD acts on these mononuclear cells to generate further more ROS and the vicious cycle continues. [9]
ROS induces uncontrolled lipid peroxidation. When lipid hydroperoxides breakdown as hydroxyalkenals, Malonyldialdehyde (MDA) is formed. MDA is used as a measure of lipid peroxidation, because it is a stable end product of lipid peroxidation.[10]

**Role of vitamin c**

Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. Ascorbic acid or vitamin C serves as a potent aqueous-phase antioxidant at low oxygen tension. This attribute of vitamin C is because of its ability to entrap free radicals present in the aqueous phase of plasma. When the capacity of ascorbic acid is exceeded, free radicals can then diffuse and oxidize proteins.

Vitamin C functions as an “electron sink”, as it donates its electrons to the free radical species, thereby converting it to less harmful forms. Reduced ascorbic acid is the biologic labile form of vitamin C. It functions as an antioxidant by donating two hydrogen atoms, a process by which the reduced form is converted to the oxidized state. In situations where ascorbic acid is consumed in quenching the free radicals, it is the reduced form which is decreased. The donation of two hydrogens atoms by vitamin C results in its conversion to dehydroascorbic acid via the ascorbyl radical intermediate. Replenishment of reduced ascorbic acid is facilitated by reduced Glutathione (GSH) which is another potent water soluble antioxidant. An overall depletion of antioxidants in hyperoxidant stress hampers the recycling of the oxidized form to the reduced form of ascorbic acid. [11,12]

**Objectives:** The study aims to evaluate the oxidative stress by measuring serum Malonyldialdehyde-(MDA) levels and antioxidant levels by measuring serum Vitamin C levels in polycystic ovarian disease and compare them with age matched healthy controls.

**Materials and Method:**

The study was carried out in collaboration with Department of Obstetrics and Gynaecology, Sassoon general hospital and private hospital, Pune. The Institutional Ethics Committee approved the study protocol. Subjects within the age group of 20 to 30 years were selected and divided into two groups. Group I consisted of 30 diagnosed female patients of PCOD. Group II comprised of 30 age matched control group with normal menstrual cycles.

**INCLUSION CRITERIA**

Patients diagnosed as PCOD by using revised diagnostic criteria (Rotterdam criteria) [13] if 2 out of 3 from the following were present: Oligo and or anovulation, Clinical and biochemical signs of hyperandrogenism, Polycystic ovaries. Female patients in the age group: 20-30 years

**EXCLUSION CRITERIA**

Diagnosed cases of diabetes mellitus, thyroid dysfunction, Cushing’s syndrome, congenital adrenal hyperplasia, hyperproloctinemia, androgen secreting tumor, renal and liver disorders.
Subjects taking medicines like ovulation induction agents, antiandrogens, antidiabetic, antiobesity, hormonal drugs and current or previous use of oral contraceptives within last 6 months, smoking and alcohol addiction. Selected patients were thoroughly interviewed in Obstetrics and gynecology department of the institute. Based on inclusion and exclusion criteria, a total of 60 subjects were selected for the present study. The study protocol was explained in detail to all the subjects and informed written consent regarding participation in the study was obtained from them. Then 5 ml of blood sample was obtained from the participant under all aseptic precautions. To assess oxidative stress, serum Malonyldialdehyde (MDA) was estimated by Buege and Aust method [14] while antioxidant level was assessed by estimation of serum vitamin C by Kyaw A method [15]. Serum MDA level and serum vitamin C levels were compared by applying unpaired t test.

Results:
Table 1 depicts the physical characteristics of the normal controls as well as the patients of PCOD. Age, height of both the groups was comparable as statistically there was no difference between them (P>0.05). Difference in mean values of age between control group (25.60±2.88 yrs) and PCOD group (25.50±2.53 yrs) was not statistically significant. (p>0.05)
Also the difference in mean values of height between control group (159.80±7.90 cm) and PCOD group (157.80±5.54 cm) was not statistically significant. (p>0.05)
But weight of the PCOD females was higher than control and the difference was statistically highly significant. (p<0.001). Mean value of weight was higher in PCOD group (62.23±5.47 kg) as compared to control group (53.90±7.94 kg)
Table 2 and Graph 1 Our study showed that serum levels of MDA was higher in PCOD group (6.96±1.29), when compared with control group (3.56±1.00) and difference in the mean value was statistically significant (p<0.001)
we also found that the serum vitamin C levels were lower in PCOD group (0.42±0.22) as compared to control group (0.93±0.44) and difference was statistically significant (p<0.001)
Table 3 and graph 2 shows that significant negative correlation (p<0.001) between serum MDA and serum vitamin C levels. This shows that an increase in MDA and decrease in vitamin C levels suggest presence of oxidative stress in PCOD patients.

Discussion:
Our study showed that the serum level of MDA (p<0.001) was significantly higher in PCOD group when compared with control group.
Similar result was obtained by Kuscu et al [16] in his study. They attributed this increase in MDA to insulin resistance and hyperglycemia in PCOD.
Yilmaz et al [17] also found significantly raised serum MDA levels in PCOD patients. This is in accordance with previous studies [18, 19, 20, 21] On the other hand, Karadeniz M et al [22] showed no statistically significant difference between PCOD and control group regarding MDA levels. This could be because they have included females with regular ovulatory cycles only. Our study also showed that the serum vitamin C levels (p<0.001) was lower in PCOD group as compared to control group. This is in accordance with previous studies [23, 24, 25]
Similarly Polak et al [26] found that the vitamin C levels were significantly lower in peritoneal fluid as well as endometrial tissue in PCOD patients.
Sekhon L et al [27] did observational study and found that up to 50-60% of recurrent pregnancy loss may be attributable to oxidative stress. Hence the antioxidant supplementation has been shown to improve insulin sensitivity and restore redox balance in patients with PCOD. In our study we found a significant negative correlation (p<0.001) between serum MDA and serum vitamin C levels. This shows that an increase in MDA and decrease in vitamin C suggest presence of oxidative stress in PCOD patients. Similar results were obtained by [28 23, 29].

Gonzales F et al [30] found that presence of oxidative stress may contribute to the chronic low levels of inflammation which often present in PCOD.

**Clinical Implications:** Role of oxidative stress in PCOD

The expression of various markers of oxidative stress has been demonstrated in normally cycling ovary. The follicular fluid microenvironment contains leukocyte, macrophages, and cytokines, all of which are known sources of Reactive Oxygen Species (ROS). ROS within the follicular fluid plays a role in modulating oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis. [31]

The probable cause of this oxidative stress in PCOD is excess of ROS in the follicle, which may overcome the follicular fluid antioxidant defense and directly damage oocytes. When the peritoneal cavity microenvironment is exposed to severe oxidative stress, the DNA of oocytes may be damaged, leading to defective fertilization. Even when fertilization is achieved, oxidative stress induced apoptosis may result in embryo fragmentation, implantation failure, abortion, impaired placentation and congenital abnormalities. [32] Excess ROS may hinder the endometrium, which normally functions to support the embryo and its development. Oxidative stress may induce luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy.[33]

**Summary and Conclusion**

Our study showed that there was an alteration in the oxidant–antioxidant profile suggestive of presence of oxidative stress in females having polycystic ovarian disease. This oxidative stress may leads to complication of disease like infertility as well as other systemic disorders e.g. diabetes mellitus, cardiovascular dysfunction, dislipidemia and endometrial carcinoma. Hence in the polycystic ovarian disease patients antioxidants can be of help to fight against these free radicals and prevent the future complication.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=30) (Mean±SD)</th>
<th>PCOD group (n=30) (Mean±SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.60±2.88</td>
<td>25.50±2.53</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.80±7.90</td>
<td>157.80±5.54</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.90±7.94</td>
<td>62.23±5.47</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

* p<0.05 statistically significant ** p<0.001 statistical highly significant
Table 2: Comparison of MDA level and Vitamin C level in the control group and PCOD group:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=30) (Mean±SD)</th>
<th>PCOD group (n=30) (Mean±SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>3.56±1.00</td>
<td>6.96±1.29</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Vit C (mg%)</td>
<td>0.93±0.44</td>
<td>0.42±0.22</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 3: Correlation between MDA and Vitamin C level in control group and PCOD group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antioxidants</th>
<th>Pearson Correlation coefficient (r)value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>Vitamin C</td>
<td>-0.480</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Graph 1: Comparison of MDA level and Vitamin C level in the control group and PCOD group:
Graph 2: Correlation between MDA and Vitamin C level in control group and PCOD group

References:
27. Sekhon L, Gupta S, Kim Y and Agarwal A. Female Infertility and Antioxidants. 84 Current Women’s Health Reviews 2010; 6: 84-95


