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ASSESSMENT OF OXIDATIVE STRESS STATUS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Endocrinology

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorders affecting 4-12% of women worldwide. It is characterized by polycystic ovaries on ultrasound scan, chronic anovulation and hyperandrogenism; allied with long term complex diseases associated with high oxidative stress. The aetio-pathophysiology of this condition is highly heterogenous involving both genes and environment.

Blood samples were collected from 400 individuals comprising 200 cases and 200 controls. Patients were selected based on Rotterdam criteria proposed by ESHRE (2003). Inter individual variation was observed among patients with respect to clinical symptoms. Forty one percent of PCOS subjects were the milder form having PCO along with oligo or anovulation and the remaining 59% were the severe form of the disease having PCO, oligo or anovulation along with clinical and/or biochemical signs of hyperandrogenism.

In order to assess the oxidative stress status in PCOS subjects the present study was carried out. The serum malondialdehyde (MDA) levels were estimated in all 400 subjects. The mean MDA levels was found to be 382.44 + 104 in patients while it was 279.97 + 94.85 in controls and differed statistically between cases and controls. Further analysis of MDA levels in milder and severe groups were 303.25 + 64.25 and 418.87 + 104.25 respectively. The mean MDA levels in milder and severe form revealed significant difference indicating higher oxidative stress in severe form of the disease compared to milder. Information on the oxidative stress status of the patient may help the clinician in the management of the condition.

KEYWORDS

PCOS, Oxidative stress, malonaldehyde, Lipid hydroperoxides

Introduction:

Polycystic ovary syndrome (PCOS) is a common endocrine disorders affecting 4% to 12% women of reproductive age worldwide [Family Paper] and also a major cause of female infertility. The heterogenous clinical features may change throughout the life span starting from adolescence to postmenopausal age and is characterized by hyperandrogenism, polycystic ovaries and chronic anovulation along with insulin resistance, hyperinsulinemia, abdominal obesity and dyslipidemia as frequent metabolic traits. These predispose the individual to serious long term complications such as hypertension, thyroid dysfunction type 2 diabetes, endometrial hyperplasia and cardiovascular disease [Ace paper].

The cause of PCOS is unknown, however several genetic, biochemical, immunological and environmental factors are implicated in the aetiopathogenesis of PCOS [Figure 1]. This disease does not show clear Mendelian inheritance however, familial clustering is observed. It is suggested that the gestational environment, lifestyle factors or both in early childhood may mediate the interaction of several genes with environmental factors to predispose the individual to develop PCOS in later life and also towards long-term risk factors associated with high oxidative stress [Micronucleus paper].

Oxidative stress refers to an imbalance between reactive oxygen species (ROS) and the antioxidant defence system, which buffers the oxidative damage. The resultant oxidative stress causes increased tissue/ cellular damage manifested by lipid per oxidation, protein oxidation and DNA damage [14-16]. The lipid peroxidation products are formed when free radicals overcome the radical-trapping-mechanisms of the body and reacts with unsaturated fatty acids resulting the formation of lipid-hydroperoxides (primary lipid per oxidation products), which are degraded to secondary lipid per oxidation products like alkanes (e.g. Ethane and pentane), aliphatic aldehydes (eg. Malonaldehyde – MDA). These products can react with DNA as well, in particular with the bases guanine and adenine. These DNA aberrations lead to erroneous transcriptions and to altered gene products. Peptide bonds are broken through the impact of MDA in proteins. Aldehydes reacts with amino groups of proteins building Schiff's - bases and as a result disturb the normal function of proteins [Figure 2].

Lipid hydroperoxides can easily pass the nuclear membrane and react with nucleic acids. Proteins are often attacked on their thiol groups changing their stereochemistry and function. Lipid hydroperoxides interfere with physical and chemical properties of the cell membrane decreases the fluidity and increases rigidity. The altered cell membrane cannot maintain their barrier function, in turn the intracellular potassium ions leak out and the enzymes are lost as well. If erythrocytes are afflicted, hemolysis takes place thereby initiate or propagate the lipid peroxidation.

Malonaldehyde (MDA) is a marker of lipid peroxidation and increases the oxidative stress status (Knight etal., 1987; Betteridge, 2000). Recent findings have documented increased oxidative stress in patients with PCOS (Sabuncu et al., 2001; Fenkci et al., 2003), which may increase the risk of type II diabetes and cardiovascular diseases in such patients. The present study was aimed to assess the oxidative stress status in PCOS women by estimating the serum MDA levels in both cases and controls. It also aimed to correlate the oxidative stress induced damage with severity of the disease.

Materials and Methodology:

A total of 400 individuals comprising of 200 patients and 200 normal healthy, ultrasound scanned controls. All the subjects were collected from Gandhi Hospital, Hyderabad and Cytomol Labs, Hyderabad. The patients were selected based on Rotterdam criteria proposed by ESHRE in 2003 [15]. Detailed information on anthropometric measures was collected through proforma. Informed consent and permission was obtained for experimentation with human subjects from the local health authorities (Institutional ethical committee, Osmania University, Hyderabad). Markers for obesity and abdominal obesity were measured by calculating body mass index (BMI) and Waist to Hip ratio (W/H) respectively. Hirsutism score was made by Ferriman-Gallway (FG) scoring system and a score of ≥ 7 was used to determine hirsuate and non-hirsuates [16]. Three milliliter of blood samples were obtained from all the subjects. Serum was separated and stored at -20°C till use for estimation of malonaldehyde levels. Information was collected from these individuals by using a proforma that included anthropometric measures, history of menstruation and signs of hyperandrogenism like hirsutism, acne, and alopecia. Based on the clinical information and the criteria of selection, the subjects were further categorized into milder and severe forms. The milder is

having only two out of three features of Rotterdam criteria whereas the severe included individuals having all the three features. The oxidative stress status was assessed in these individuals through estimation of serum malonaldehyde levels according to the method described by Nadiger et al., 1987 [17].

Estimation of Malondialdehyde:
In a clean centrifuge tube add 1440 µl of 10% Trichloroacetic acid, 600 µl of 0.67% Thiobarbituric acid, 400 µl of distilled water followed by 200 µl of serum sample. Mix all the contents vigorously by using a vortex machine, cover the tube with aluminum foil to prevent evaporation and place it in a boiling water bath till the color changes from milky white to light pink. Cool the tubes to room temperature and centrifuge at 5000 rpm for 10 minutes to get clear supernatant. Take 1 ml of the supernatant and read OD at 520 nm calorimetrically using distilled water as blank.

Statistical Analysis:
All the values were expressed as mean ± SD. For statistical comparisons between the patients and control group, t – test for independent samples was used. . Two-tailed p values less than 0.05 were considered to be significant.

Results:
The data analysis of 400 individuals involved in the present study demonstrated an age range of 13 - 40 years in cases while 16 - 46 years in controls with a mean age of 24.78 ± 4.39 and 24.91 ± 4.81in cases and controls respectively at the time of sampling. The mean age at onset (AAO) of the clinical symptoms in patients was 16.18 ± 4.43 years. Nearly 50% of our cases are overweight or obese with BMI ≥ 23 kg/m^2. A significant difference with respect to BMI and WHR was observed between the groups ($p < 0.05$). Thirty three percent of cases showed hyperandrogenism in 33 % in the form of hirsutism, acne, alopecia and premature pubarche. Twenty three percent demonstrated acanthosis, a marker for insulin resistance while 14 % of patients showed both hyperandrogenism and acanthosis [Figure 2]. The mean MDA levels were found to be 382.44 ± 104 ηM/ml and 279.97 ± 94.84 ηM/ml respectively in patient and control group and the difference was found to be statistically significant ($p < 0.05$). The characteristics of PCOS and controls along with mean MDA levels are presented in Table 1.

Inter-individual variation was observed among patients with respect to clinical symptoms. Forty one percent of cases were having PCO along with menstrual disturbance; while the remaining 59% were the showing all the three characteristics of Rotterdam criteria. In this regard, we further categorized the patients into milder and severe groups based on the severity of the disease. The clinical features revealed a significant difference with respect to BMI, W/H, AAO and DOD between the groups. However, age and AAM showed insignificant difference. The mean MDA levels were found to be 303.25 ± 64.25 ηM/ml in milder PCOS group compared to 416.91± 104.11 ηM/ml in the severe group and differed significantly between the groups. The duration of disease was longer in severe compared to the milder group. The results are shown in Table 1

Discussion:
PCOS is a common complex disorder and the complexity of the underlying genetic model as well as potential gene-gene and gene-environment interactions pose a difficulty for genetic analysis (ut etal). Association of several candidate genes with PCOS susceptibility suggests genetic predisposition in the aetiopathogenesis of PCOS. However, few studies also suggest oxidative stress as a contributing factor towards PCOS. Hence, the present study was aimed to estimate the serum MDA in PCOS cases and controls. Analysis revealed a high MDA levels in cases compared to controls indicating a high oxidative stress in the causation of PCOS. The rise in MDA could be due to increased reactive active species (ROS) generation leading to high oxidative stress induced damage to various cells and tissues resulting in DNA damage as well as signaling errors that appears to play an important role in causation of PCOS. These observations were supported by other studies [Yildirim B et al 2007, 17-20]. The chronic low level inflammation observed in PCOS cases as assessed by proinflammatory genotypes appears to be one of the major contributing factors to induce net oxidative stress leading to insulin resistance and hyperandrogenism in PCOS. The decreased antioxidant status may contribute to increased risk of cardiovascular disease in women with PCOS, in addition to other risk factors like insulin resistance, diabetes, hypertension, obesity and dyslipidemia. Hence, treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage.

Competing Interests: None

Acknowledgements:
We thank all the patient and controls for their active participation to carry out the desired work.

Figure1: Aetio-pathophysiology of Polycystic Ovary Syndrome

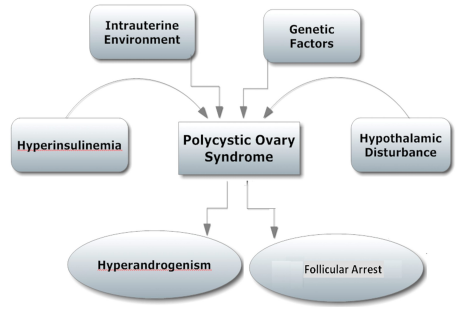


Figure 2: Schematic representation of release of excess reactive oxygen species and its consequent tissue damage

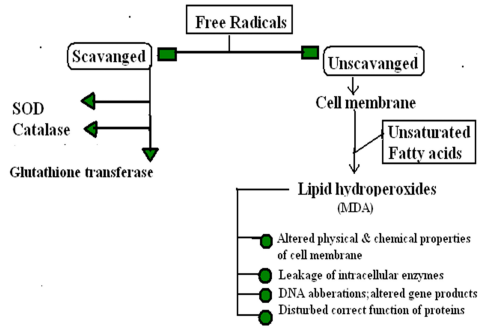
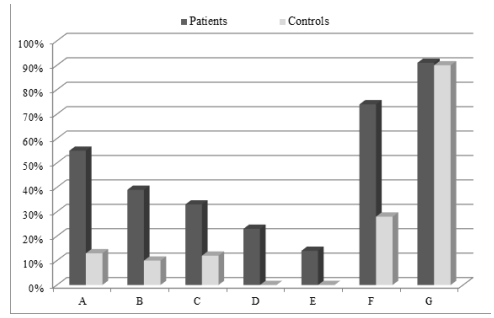


Figure 3: Distribution of anthropometric measures, clinical features, family history of complex diseases and diet in PCOS probands and controls



A: Higher BMI, **B:** Higher WHR, **C:** Hyperandrogenism, **D:** Acanthosis, **E:** Hyperandrogenism + Acanthosis, **F:** Family history of complex diseases, **G:** Diet (Non-vegeterian)

Table 1: Subject characteristics of the study group

Variables	Total Patients	Total Controls	p-value	Mild Form	Severe Form	p-value
Age (years)	24.29 ± 4.05	26.05 ± 5.59	0.0081*	23.42 ± 3.73	24.91 ± 4.65	0.099
BMI (Kg/mt2)	25.89 ± 5.65	22.49 ± 4.38	0.0001*	25.11 ± 5.76	28.88 ± 5.96	0.002*
W/H ratio	0.79 ± 0.06	0.77 ± 0.05	0.0057*	0.78 ± 0.05	0.8 ± 0.06	0.089
AAM	12.42 ± 0.91	12.30 ± 0.91	0.317	12.69 ± 0.84	12 ± 1.96	0.048*
AAO	13.74 ± 2.72	…….	------	14.73 ± 3.24	13.30 ± 2.19	0.007*
DOD	10.20 ± 5.30	…….	------	8.69 ± 4.95	11.69 ± 5.54	0.007*
MDA	382.44 ± 104	279.97 ± 94.85	0.0001*	303.25 ± 64.25	416.91 ± 104.11	0.0001*

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