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Original Article

Biochemistry Section

Oxidative Stress in Non-Obese Women with Polycystic Ovarian Syndrome

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is one of the most common endocrine diseases of women. Oxidative stress is an important component of the cardio-metabolic risk seen in these women. Oxidative stress has been reported in obese PCOS women. This study is taken up to study oxidative stress in non-obese PCOS women.

Materials and Methods: Twenty five non-obese women with PCOS attending the Endocrinology outpatient Department of Sri Venkateswara Institute of Medical Sciences, Tirupati, India along with 25 healthy BMI matched controls were included in the study. The changes in the lipid peroxidation products (MDA), and total anti oxidant capacity (FRAP) as an index of anti oxidant status along with fasting glucose, insulin and uric acid levels were measured in both groups. Insulin resistance was evaluated by using homeostasis model assessment for insulin resistance [HOMA-IR)= [FPG (mg/dl) × insulin (mIU/L)]/ 405] in both groups.

Results: Serum MDA and uric acid levels were increased in the study group compared with controls and FRAP levels were decreased in the study group compared to controls though statistically insignificant.

Conclusion: Oxidative stress is also present in non-obese women with PCOS. Oxidative stress further increases the CVD risk in these women.Correcting oxidative stress with antioxidants along with monitoring the antioxidant status using a simple assay like FRAP could have a beneficial effect on oxidative stress induced insulin resistance and hyperandrogenism seen in these women.

Keywords: Cardiovascular risk, FRAP, Insulin resistance, Malondialdehyde

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder of unknown aetiology affecting 5-10% of women of reproductive age [1]. It is a disorder that affects the reproductive, endocrine and metabolic functions and is the leading cause of chronic anovulation leading to infertility [2]. Also, it is associated with cardiovascular risk factors including obesity, insulin resistance, dyslipidemia, endothelial dysfunction and presence of metabolic syndrome [3, 4]. The mechanisms underlying oxidative stress in PCOS are not fully understood, but recent studies strongly suggest that IR plays a pivotal role in the pathogenesis of PCOS and encourages oxidative stress [5].

Oxidative stress occurs due to an imbalance between the production of ROS and the antioxidant defence system. In women, Oxidative stress might play a role in infertility [6]. Oxidative stress also impairs insulin action, as seen in type 2 diabetes mellitus, and this impairment might be due to several factors, such as membrane fluidity alterations, decreased availability of nitric oxide and increased intracellular calcium content [7].

Study of oxidative stress in PCOS is important since cardiometabolic risk factors along with insulin resistance seen in these women are associated with endothelial dysfunction [8] an early reversible marker of atherosclerosis. Oxidative stress is also known to independently contribute to endothelial dysfunction [9]. All these could together lead to increased cardiovascular risk in these women.

Factors likely to contribute to increase oxidative stress in PCOS include obesity and insulin resistance commonly seen in these women [3,4]. We have previously shown that body iron stores as reflected by serum ferritin levels are increased in non-obese women with PCOS [10]. It has previously been proposed that the increase in ferritin levels can be due to a response to oxidative stress [11], since ferritin sequesters unbound iron and prevents oxidative damage to tissues [12].

Studies showing oxidative stress in PCOS women have reported conflicting results. A few studies have reported increased oxidative stress in obese [13,14] as well as in lean PCOS women [15-17]. However, a recent study done in Chinese population reported increased oxidative stress only in obese PCOS women and not in lean PCOS women [18]. There is one study in Indian women with PCOS which was done in obese individuals [19]. Hence the present study was undertaken to assess the oxidative stress in non-obese PCOS women from South India.

MATERIALS AND METHODS

In the present study, 25 PCOS patients attending the Endocrinology outpatient Department of Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India aged 20-38 years and diagnosed as PCOS based on National Institutes of Health (NIH) consensus 1990 criteria [20] were included after written informed consent. The inclusion and exclusion criteria for the study subjects are presented in [Table/Fig-1]. Twenty five age-matched healthy females from among the hospital staff were taken as controls. The study was approved by institutional ethical committee.

Anthropometric measurements including height, weight and body mass index (BMI) and systolic and diastolic blood pressures of all subjects were recorded. Five ml of venous blood sample after over night fast were collected in a plain bulb from subjects of both groups. The sample was then centrifuged at 3,000 rpm for 15 min and serum obtained was stored at -80ºC until further analysis. Plasma glucose levels were estimated on Beckman C X 9 fully automated analyzer

Inclusion criteria	Exclusion criteria
Study group: PCOS diagnosed according to the NIH consensus 1990 criteria 1. Oligomenorrhoea (≤ 9 menses/year) or amenorrhea (no menstrual periods for 3 or more months), 2. Hyperandrogenism and/or hyperandrogenaemia	Related disorders with similar presentation like: 1. Hypothyroidism [thyroid stimulating hormone (TSH) >5 mIU/mL)], 2. Hyperprolactinaemia (serum prolactin > 100 ng/mL), 3. Cushing's syndrome (cortisol > 2 µg/dL), 4. Adrenal hyperplasia and androgen secreting tumours (testosterone levels greater than 3 times the upper reference limit associated with relevant clinical features). 5. Women with virilization, pregnancy, those on oral contraceptives, glucocorticoids, anti- androgens, ovulation inducing agents, antidiabetic drugs or antiobesity drugs or other hormonal drugs during the previous 6 months.
Control group: Age matched, with absence of: 1. menstrual irregularities, 2. hirsutism and 3. major medical illness	

[Table/Fig-1]: Inclusion and exclusion criteria

using commercially available kits. MDA was measured as thiobarbituric acid reactive substances (TBARS) [21]. Total antioxidant capacity was measured as ferric acid reducing ability of plasma (FRAP) by UV spectrophotometry [22]. Serum insulin levels were measured by ELISA using DIA source Ins-Easia kit from DIAsource immunoassays (S.A.-Rue de l'Industrie, 8-B-400 Nivelles-Belgium) on CHEMWELL analyzer. Insulin resistance as per the homeostasis model assessment method (HOMA-IR) was calculated using to the formula [FPG (mg/dl) × insulin (mIU/L)]/ 405 [23].

STATISTICAL ANALYSIS

All values were expressed as mean ± standard deviation for normally distributed data and as median (inter quartile range) for skewed data. Differences between the two groups were analyzed using Mann-Whitney U test. A p-value less than 0.05 was considered as statistically significant. Statistical analysis was done by using Microsoft excel spread sheets and SPSS for windows version 11.5 (SPSS, Inc; Chicago IL).

RESULTS

[Table/Fig-2] shows the baseline characteristics in the study group and control group. Both groups were similar in terms of BMI, fasting blood glucose, systolic blood pressure (SBP) and diastolic blood pressure (DBP).

[Table/Fig-3] shows the levels of various oxidant and antioxidant parameters like MDA, FRAP and Uric acid in the study group and control group. MDA and Uric acid levels were found to be significantly increased in the study group when compared to control group (p=0.02, p=0.001 respectively). FRAP found to be significantly decreased in the study group compared to control group (p = 0.09).

Characteristics	Cases	Controls	P-value
Age (years)	22.52 ± 5.10	25.36 ± 5.52	0.065
Body Mass Index (BMI) (Kg/m^2)	24.40 ± 2.07	25.10 ± 3.01	0.345
Systolic Blood Pressure (mmHg)	110.80 ± 9.09	113.60 ± 5.68	0.199
Diastolic Blood Pressure (mmHg)	73.20 ± 6.27	72.40 ± 4.35	0.603
Testosterone (ng/dL)	2.06 ± 0.45	0.78 ± 0.29	0.001*
Fasting Blood Glucose (FBS) (mg/dL)	94.24 ± 6.87	91.52 ± 5.27	0.123
Insulin µIU/ml)	14.96 (10.92-28.40)†	5.80 (4.99-18.48)	0.024*
Insulin resistance (HOMA-IR)	3.63 (2.56-6.02) †	1.34 (1.06-4.38)	0.020*

[Table/Fig-2]: Baseline characteristics in women with PCOS and controls
* =Statistically significant; †= median (inter-quartile range)

Parameters	Cases (n=25)	Controls (n=25)	p-value
Malondialdehyde(MDA) (µmol/L)	3.22 ±1.53	2.42 ± 0.55	0.02*
Ferric Reducing Ability of Plasma (FRAP) (mmol/L)	1.45 ± 0.36	1.64 ± 0.41	0.09
Uric acid(mg/dL)	5.68 ± 1.29	4.18 ± 0.79	0.001*

[Table/Fig-3]: Oxidant and antioxidant parameters in cases and controls
* Statistically significant

DISCUSSION

In the present study MDA levels were found to be significantly increased in study group compared to control group (p=0.02). This is in agreement with previous reports [15-17] which have reported increased oxidative stress even in lean PCOS women. Oxidative stress was previously linked to obesity commonly seen in these women [13,18]. However, a few other studies have found increase in oxidative stress even in lean PCOS women [15-17].

PCOS women often have insulin resistance [24]. Hyperglycemia has been thought to play a role in inflammation through production of tumor necrosis factor-α (TNF-α) from mononuclear cells (MNCs). These MNCs also produce ROS resulting in cellular damage, thereby activating nuclear factor-κB, a proinflammatory transcription factor that promotes the transcription of TNF-α, a known mediator of insulin resistance [25,26]. Insulin resistance seen in our cases [Table/Fig-1] supports this finding. This oxidative stress has also been implicated as a causal factor for hyperandrogenism in these women [24]. However, recent experimental studies suggest that androgen excess in PCOS women increases leukocytic ROS generation, p47phox gene expression, and plasma TBARS to promote oxidative stress in the presence of hyperglycemia in lean healthy reproductive-age women. Thus in PCOS, hyperandrogenism may be the progenitor of diet-induced oxidative stress independent of obesity or excess abdominal adiposity [27]. This could explain the presence of oxidative stress even in the absence of obesity in our PCOS women.

Total anti oxidant Status (TAOS) is sensitive to changes in plasma antioxidant levels and degrees of oxidative stress. In the present study we estimated FRAP as a measure of total antioxidant capacity which represents the effect of the reducing power of plasma constituents, contributed by low molecular weight antioxidants of a hydrophilic and hydrophobic character especially vitamin C, vitamin E, bilirubin and uric acid and thus gives more biologically and clinically relevant information on antioxidant capacity [28].

In most of the studies TAOS was found to be decreased in PCOS women compared to controls [13,16,19]. However, in a study from Turkey, total antioxidant status was found to be increased in non-obese women with PCOS [29]. In the present study, FRAP levels were found to be decreased in study group when compared to controls but statistically not significant. The major determinant of plasma FRAP is uric acid, accounting for 60% of the total FRAP activity. In the present study serum uric acid concentrations were found to be significantly increased in the study group compared with controls (p<0.001) and this could be the probable reason for our finding of nonsignificant decrease in FRAP levels. Those studies who reported decrease in FRAP levels did not study uric acid levels. Higher uric acid concentrations have also been previously reported in PCOS women [30]. This could probably be the reason for the lesser degree of decrease in FRAP in the present study. Hyperuricemia is also a component of the metabolic syndrome commonly found in PCOS women.

CONCLUSION

The findings of the present study indicate the presence of oxidative stress as evidenced by an increase in MDA levels and a decrease in FRAP levels in non-obese PCOS women. PCOS is associated with dyslipidemia, metabolic syndrome and cardiovascular risk factors especially elevated triglycerides which serve to increase the substrate for free radicals which are not neutralized by the defective antioxidant system. As a consequence oxidative stress may enhance lipid peroxidation and further contribute to the development and progression of atherosclerosis. Correcting oxidative stress with antioxidants could have a beneficial effect on oxidative stress induced insulin resistance and hyperandrogenism seen in these women.

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