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Analysis of Oxidative Stress Status Through MN Test and Serum MDA Levels in PCOS Women

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Abstract: Polycystic Ovary Syndrome (PCOS) is a multifactorial reproductive healthcare problem affecting 4-12% of women and a leading cause of female infertility worldwide. The potential genetic contributors of PCOS are unclear. However, over the past decade emerging evidence has shown that increased Oxidative Stress (OS) and decreased antioxidant status were often linked with PCOS. The present case-control study was aimed to assess the reactive oxygen species induced OS in women from South India. A total of 164 individuals comprising of 89 patients and 75 controls were enrolled in the present study. For all the subjects, the frequency of micronucleated cells (MNC) in epithelial samples and serum Malondialdehyde (MDA) levels were estimated to assess genomic instability and cytotoxicity respectively. A statistically significant difference between the groups were identified with respect to Body Mass Index, Waist to Hip Ratio, luteinizing hormone and prolactin levels (<0.05), however the mean follicle stimulating hormone was not different between the groups ($p = 0.055$). The frequency of MN cells (5.89 ± 4.86 vs. 2.24 ± 2.01) and mean serum MDA (360.84 ± 87.08 vs. 301.70 ± 82.82) levels were considerably higher in patients than controls ($p = <0.0001$), furthermore, a positive correlation was observed between MNC and MDA levels in patients ($r = 0.349$, $p = 0.0008$) and not in controls ($r = 0.104$, $p = 0.37$), suggest high OS in PCOS women. Therefore, MN assay and serum MDA levels may serve together or individually as biomarkers of OS in PCOS women.

Key words: Polycystic ovary syndrome, micronucleus assay, oxidative stress, malonaldehyde

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder, characterized by chronic anovulation, hyperandrogenism and polycystic ovaries on ultrasound scan, a leading cause of female infertility (Stephen, 1995). It affects 4-12% of women of reproductive age worldwide (Hahn *et al.*, 2005). Inter-individual variation is commonly observed with respect to clinical features changing throughout the life span, starting from adolescence to postmenopausal age. The pathophysiology of PCOS remains unknown, however suggests gestational environment or lifestyle factors or both in early childhood may mediate the interaction of several genes with environmental factors to predispose the individual to develop PCOS (Escobar-Morreale *et al.*, 2005). Emerging evidence has suggested that Reactive Oxygen Species (ROS) induced Oxidative Stress (OS) may play a significant role in the manifestation of insulin resistance and hyperandrogenism, the key features of PCOS (Rosenfield, 1999; Dunaif, 1997; Sahin *et al.*, 2004).

Oxidative stress that arises due to an imbalance between generation of ROS and antioxidant defense has

been linked to a number of disease states such as cardiovascular diseases, aging, cancers including PCOS (Yesilada *et al.*, 2006). The resultant OS causes increased tissue/cellular damage manifested by lipid per oxidation, protein oxidation and induces formation of bone marrow micronucleus (MN) (Reddy *et al.*, 2011; Simic, 1994).

The present study was aimed at investigating the oxidative stress status through MN assay and estimation of serum MDA levels in women from South India.

MATERIALS AND METHODS

Subjects: The study was carried out in 164 women comprising of 89 patients and 75 controls. Samples were obtained from Government Maternity Hospital, Petlaburz, Hyderabad, India. Patients were selected based on Rotterdam criteria proposed by Rotterdam ESHRE/SRM-Sponsored PCOS Consensus Workshop Group (2004). Informed consent was taken from subjects prior sample collection. Ethical clearance was obtained from local ethical committee (Osmania University, Hyderabad, India). Detailed information on clinical and anthropometric measures was collected through

proforma. Obesity was measured by calculating Body Mass Index (BMI); waist/hip ratio (WHR) was used as a marker for abdominal obesity and a value of ≥ 0.8 was considered as obese. Ferriman-gallway (FG) score of ≥ 7 was used to determine hirsutism (Ferriman and Gallway, 1961). The inclusion criteria for controls was healthy, ultrasound scanned normal fertile women with no signs of menstrual dysfunction or history of infertility. Exfoliated cells of buccal epithelium and 1 mL of blood sample were obtained from each individual for micronucleus assay and to estimate serum MDA levels (Nadiger *et al.*, 1987), respectively. Serum was separated from the blood and stored at -20°C till use for MDA analysis and buccal samples were processed further for micronucleus test.

Micronucleus Test (MNT): Micronuclei are formed as a result of chromosomal breakage or spindle damage and are identical to the main nucleus, varying in their diameter and not linked to the main nucleus (Knight *et al.*, 1987; Jahan *et al.*, 2009; Betteridge, 2000; Sabuncu *et al.*, 2001). For MN assay, exfoliated cells were collected by the help of ice-cream stick and suspended in a centrifuge tube containing 10 mL of buffer solution (0.1 mM EDTA, 0.01 M Tris HCl and 0.02 M NaCl). The tubes were centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the step was repeated thrice. Finally 3/4th of the supernatant was removed and the pellet containing 1/4th of the buffer was dropped on a chilled slide by the help of a dropper. After air drying, the slides were fixed by placing them in a fresh fixative solution containing methanol and glacial acetic acid in the ratio of 3:1. Slides were air dried and stained with giemsa. Finally, the Micronucleated Cells (MNC) were analyzed under light microscope by scoring 1000 cells and the number of MNC for each individual was recorded (Fig. 1).

Serum Malondialdehyde (MDA) levels: In a clean centrifuge tube 1.44 mL of 10% Trichloroacetic acid, 0.6 mL of 0.67% thiobarbituric acid, 0.4 mL of distilled water and 0.2 mL of serum sample were added and mixed vigorously by using a vortex machine. The tube was covered with aluminum foil to prevent evaporation and placed on a boiling water bath till the color changes from milky white to light pink. Finally, the tube was cooled to room temperature and centrifuged at 5000 rpm for 10 min to get clear supernatant. One milliliter of the supernatant was collected and OD was read at 520 nm calorimetrically using distilled water as blank.

Statistics: All the values were expressed as Mean \pm SD. For statistical comparisons between the patients and

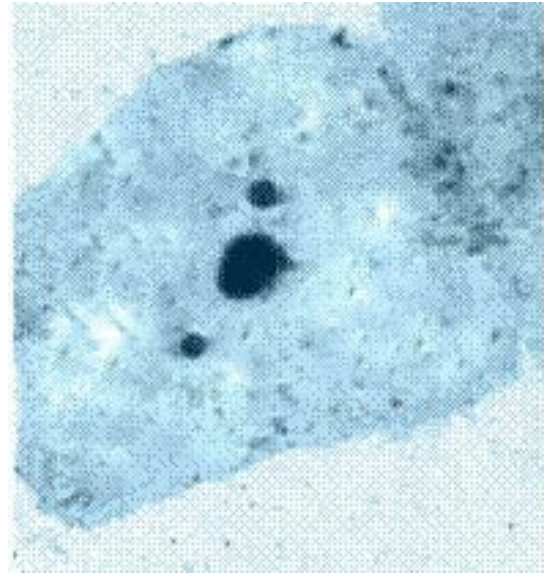


Fig. 1: Buccal epithelial cell with micronuclei

control group, t-test for independent samples was used. A two-tailed p -value < 0.05 was considered to be significant. Correlation analysis was carried out between the frequency of micronucleated cells and the MDA levels. The SPSS package (17th version) was used for statistical analysis.

RESULTS

Data analysis on a total cohort of 164 individuals revealed that the mean age of the patients and controls at the time of sample collection was 24.67 ± 5.28 years and 25.22 ± 5.12 years, respectively. The results of anthropometric measures, biochemical characteristics and OS analysis of patients and controls were given in Table 1.

Analysis of anthropometric measures revealed that BMI and WHR was statistically significant between the patients and controls ($p < 0.05$); however, age and Age at menarche (AAM) did not differ significantly between the groups ($p > 0.05$). The levels of LH and prolactin were also significantly ($p = < 0.001$, $p = < 0.0001$, respectively) higher in patients than in controls, whereas the levels of FSH did not show variation between the groups ($p > 0.05$).

The frequency of micronucleated cells were significantly ($p = 0.042$) higher in women with PCOS than in controls. The mean MDA levels were found to be significantly higher ($341.88 \pm 67.08 \mu\text{M mL}^{-1}$) in patients than in controls ($307.69 \pm 49.65 \mu\text{M mL}^{-1}$). In addition, a strong positive correlation was observed between the

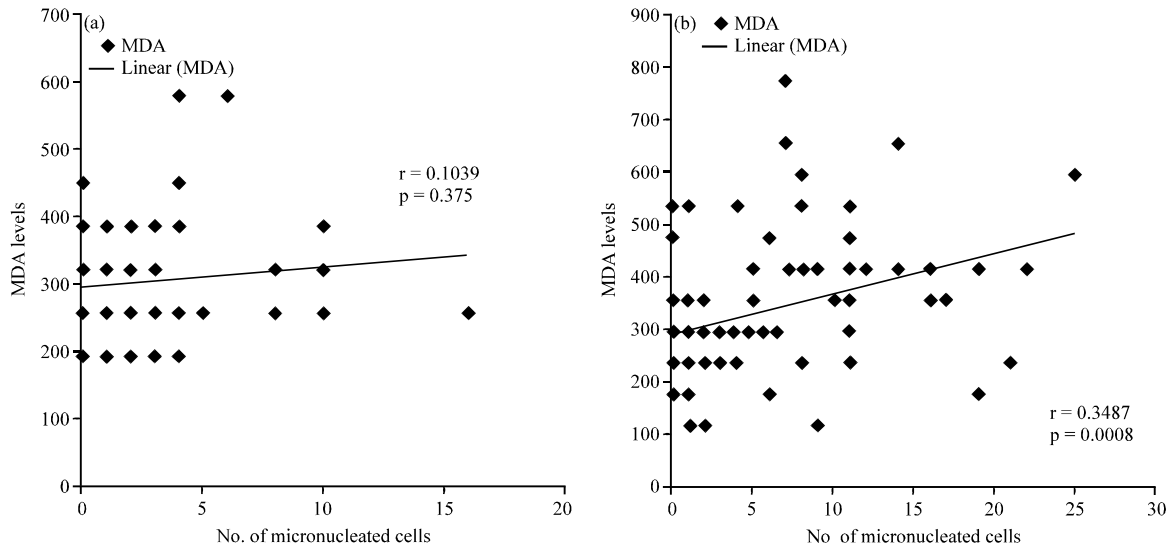


Fig. 2(a-b): Correlation between micronucleated cells and MDA levels in (a) Controls and (b) Patients

Table 1: Demographic, biochemical and OS status of patients and controls

Characteristics	Patients (n = 89)	Controls (n = 75)	p-value
Age (years)	24.67±5.28	25.22±5.12	0.501
BMI (kg m ⁻²)	25.47±4.14	21.17±1.89	<0.0001*
WHR	0.78±0.05	0.74±0.03	<0.0001*
AAM (years)	12.53±1.12	12.28±0.84	0.113
Hormonal profile			
LH (mU mL ⁻¹)	8.91±2.96	5.98±1.03	<0.0001*
FSH (mU mL ⁻¹)	6.27±2.13	5.74±1.16	0.055
Prolactin (ng mL ⁻¹)	11.98±5.82	7.69±2.36	<0.0001*
Oxidative stress			
MNC frequency (per 1000 cells)	5.89±4.86	2.24±2.01	<0.0001*
Malondialdehyde levels (̑M mL ⁻¹)	360.84±87.08	301.70±82.82	<0.0001*

Quantitative data are presented as Mean±SD, BMI: Body mass index, WHR: Waist to hip ratio, AAM: Age at menarche, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, MN: Micronucleus, *p-value<0.05

frequency of MN cells and MDA levels in the patient group (p<0.05) however, no correlation was seen in the controls (p>0.05) (Fig. 2).

DISCUSSION

PCOS is a common complex disorder whose etiopathogenesis is not well understood. However, genetic, biochemical, immunological factors and environment are implicated in the causation of PCOS. The inherent genetic susceptibility may include oxidative stress related candidate genes which may contribute to increased tissue/cellular damage. Several other studies of TNF alpha gene polymorphisms have shown to be associated with PCOS, a proinflammatory condition (Gonzalez *et al.*, 2006a; Kirwan *et al.*, 2001).

The present study aimed to assess the simple and inexpensive OS prognostic markers in PCOS patients and controls. Micronucleated cell frequencies in exfoliated cells provide an index of accumulated genetic damage occurring during the life span of these cells. The inter-individual variation with respect to MNC frequency is suggested to be explained by lifestyle factors, including environmental exposure, or individual susceptibility factors (Huber *et al.*, 1992). The oxidative stress that arises due to imbalance between Reactive Oxygen Species (ROS) and the antioxidant defense system buffers the oxidative damage and induce breakage that lead to the formation of micronucleus.

Significantly higher frequencies of MNC and elevated serum MDA levels in our study among patients than controls are in consistent with previous findings (Yesilada *et al.*, 2006; Parker *et al.*, 1980). Further, a noticeable correlation was seen between MNC and MDA levels among the patients supports the findings of Frank Gonzalez (Gonzalez *et al.*, 2006b). Our results are suggestive of OS stress related genetic instability and cytotoxicity in PCOS women.

CONCLUSION

In conclusion, the above observations are indicative of potentiality of these two parameters as prognostic biomarkers together or individually which may help the clinicians in the treatment and management of the condition.

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