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RESEARCH ARTICLE

GENETIC INSTABILITIES AND OXIDATIVE STRESS IN CHILDREN BORN TO PCOS MOTHERS

***Sreeja CK[1], Swapna C Senan[2], Deepthi T[2], Akhila V[3], Viji Krishnan[4], Sharmila K[4] and Dinesh Roy D[3]**

1. Department of Physiology, Jubilee Mission Medical College and Research Institute, Thrissur-680005, India
2. Department of Medical Microbiology, Sree Gokulam Medical College, Thiruvananthapuram- 695607
3. Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram- 695 024
4. Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur-680005

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Corresponding Author

Sreeja CK

Abstract

Polycystic ovary syndrome (PCOS) is a complex and frequent disorder with a heterogeneous clinical presentation varying throughout life. Children born to PCOS mothers are considered to be at risk for early insulin resistance, leading to development of PCOS and metabolic abnormalities in childhood and adolescence. Pregnant women with PCOS exhibit a significant increase in androgen concentrations during pregnancy, which could provide a potential source of androgens to the foetus, and is perhaps another mechanism to explain the higher prevalence of low birth weight newborns in PCOS mothers. Thirty five children born to polycystic ovarian syndrome mothers were selected as study subjects and 20 children born to normal mothers were selected as control for this study. The goal of the present study was to evaluate the genetic instabilities and oxidative stress, if any, in children born to PCOS mothers. The oxidative stress biomarker malondialdehyde (MDA) value and the micronuclei frequency was significantly elevated in study subjects as compared with that of control subjects. The data indicate that DNA damage and susceptibility of DNA to oxidative stress are increased in children born to PCOS mothers. This suggests that oxidative stress may be one of the important causes for abnormal endometrial environment with poor embryo receptivity in PCOS patients.

INTRODUCTION

Polycystic ovary syndrome (PCOS), first reported by Stein and Leventhal, is now recognized as a common, heterogeneous, heritable disorder affecting women throughout their lifetime. PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. PCOS adversely affects endocrine, metabolic, and cardiovascular health (Susan and Kristen, 2014). Common clinical manifestations include oligomenorrhea, hyperandrogenism, acanthosis nigricans, insulin resistance, reproductive aberration and obesity (Joo et al, 2010).

PCOS occurs in 4-8% of women during their reproductive years, and it is the most frequent endocrine disease in women (Khan et al, 2006). Approximately one in 15 women experiences PCOS (Norman et al, 2007). The prevalence of obesity in clinical series of patients with PCOS ranges from 30 to 75% (Ehrmann, 2005) and in as many as 28% of overweight and obese women with PCOS is also present (Blasco et al, 2006).

Oxidative stress results from an increase in reactive oxygen species (ROS), as the antioxidant capacity of the cells to scavenge and remove these free radicals decreases. ROS is the byproduct of the process of respiration taking place in the mitochondria (Ruder et al, 2009). Commonly, ROS molecules interact with proteins, lipids, carbohydrates, or DNA molecules within cells, causing damage to cellular structures including cell membranes and genetic material (Corton et al, 2008). The imbalance of ROS can eventually lead to epigenetic differences and changes in cellular pathways and transcription factors. In women, oxidative stress might play a role in infertility (Agarwal et al, 2008).

Infertility in PCOS patients was related to the effect of follicular fluid's oxidative stress levels on the meiotic spindle formation in the oocyte. Fertilization rates and embryo quality were found to be decreased in these women. Many studies indicate that elevated ROS in the follicular fluid tends to limit the fertilization potential of oocytes, through disruption of meiotic spindle formation (Chattopadhayay et al, 2010). All women experience increasing insulin resistance and abdominal adiposity along with chronic inflammation and dyslipidemia with age and a specific increase in LDL across the menopausal transition (Matthews et al, 2009).

Recent studies have shown that PCOS is associated with hyperinsulinemia and insulin resistance and may lead to cardiovascular diseases. In young women with PCOS, multiple risk factors for CVD including metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), dyslipidemia, abdominal obesity and hypertension were reported. Various studies demonstrated the presence of chromosomal damage in circulating cells of patients with CAD using the Cytokinesis-block micronuclei (CBMN) assay (Simon et al, 2011; Andreassi et al, 2011).

Unsaturated fatty acid peroxidation is a radical chain reaction initiated by the abstraction of a hydrogen atom from a methylene group of the fatty acid chain. Products of lipid peroxidation reactions have been widely employed as biomarkers for Oxidative Stress (OS). MDA, produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a good biomarker (Abuja and Albertini, 2001).

The polycystic ovarian syndrome is a familial disorder, but the genetic basis of the syndrome remains controversial. Pregnant women with PCOS exhibit a significant increase in androgen concentrations during pregnancy, which could provide a potential source of androgens to the foetus, and is perhaps another mechanism to explain the higher prevalence of low birth weight newborns in PCOS mothers (Sir-Petermann et al, 2002).

The offspring of PCOS mothers may represent a high risk group with a great potential for early clinical intervention. In this regard, a treatment programme, including diet, exercise and insulin sensitising agents before pregnancy in those PCOS women who desire fertility, and diet and exercise in those who become pregnant, may constitute a timely therapeutic approach. Following this approach, the incidence of pregnancy complications such as gestational diabetes and pregnancy-induced hypertension observed in our PCOS patients was lower than that reported in the literature (Lesser and Garcia, 1997; Urman et al, 1997). Moreover, as previously reported (Urman et al, 1997), the incidence of pregnancy complications is higher in obese PCOS patients.

There is now a larger focus on the management of the metabolic outcomes of PCOS, essentially through lifestyle intervention to achieve weight loss and developing physical activity. There are only few studies concerning about the oxidative stress in PCOS patients especially in heir offsprings. Hence, the present study was undertaken to evaluate genetic instabilities and oxidative stress in children born to PCOS mothers.

Materials and Methods

Thirty five children born to polycystic ovarian syndrome mothers were selected for this study. The samples were referred from various maternity centers of Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. 20 subjects were also selected as control for this study. Detailed, social and clinical characteristics were recorded using proforma. In this study Peripheral blood lymphocyte culture (PBLC), Cytokinesis Block Micronuclei (CBMN) Assay and Malondialdehyde test (MDA) was carried out in each sample for the research study. PBLC was performed to analyze chromosomal aberrations. Cytokinesis Block Micronuclei (CBMN) Assay was performed on each sample by using cytochalasin – B for quantitating the extent of somatic DNA damages and MDA test was conducted to analyse oxidative stress in children born to PCOS mothers.

Collected eight ml of blood sample by venepuncture and transferred 3 ml of blood into sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay.

The whole blood was added to 5 mL RPMI 1640 supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, 15% fetal bovine serum and 1% phytohemagglutinin. CBMN assay was carried out by the method of Fenech (1993). Cytochalasin B was added to the cultures at a final concentration of 4.5 µg/ mL (Sigma) after 44-h incubation stimulation with phytohaemagglutinin. Cells were harvested after 72-h incubation, and they were treated with a hypotonic solution (0.075 M KCl) for 1 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air-dried and stained with Giemsa. Micronucleated cells were analyzed under light-microscopy at 100 X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

The remaining five ml of blood was transferred into a plain tube. Blood was allowed to clot, serum separated immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer. The level of the serum lipid peroxide marker, malondialdehyde (MDA) was determined using thiobarbituric acid as main reagent and measuring these values on photoelectric colorimeter at 540nm.

Results

Table 1 Distribution of Mean CBMN frequency and MDA value among the study and control subjects

Variables	Number	Mean CBMN Frequency	Mean MDA Value
Study Subjects	35	11.95	1.21
Control Subjects	20	10.13	0.93

Thirty five children born to PCOS mothers and 20 normal healthy children were selected and analyzed for the study. The mean CBMN frequency of study subjects was observed as 11.95 and that of the control subjects as 10.13 which showed a statistically significant difference. The MDA value of the study subjects was 1.21 and that of the control subjects was 0.93. The study frankly demonstrated that MDA level increased among the study subjects than the control subjects (table 1).

The age of children born to PCOS mothers were ranged from 1 to 9 and it was further grouped in to 1 to 3, 4 to 6, and 7 to 9. Highest mean CBMN frequency (13.6) was observed in age group ranged from 7 to 9. The mean CBMN frequency was observed high in both males and females. Age of father ranged from 26 to 45. The highest mean CBMN frequency (12.1) was observed among the paternal age group 41 to 45. Age of mother ranged from 26 to 40. The highest mean CBMN frequency was observed in mothers belonging to 36 to 40 years of age. Majority of the study subjects belonged to rural (51.42%) followed by urban area (34.48%) and coastal area (14.28%). The highest mean CBMN frequency was observed in coastal area (12.1).

Regarding clinical conditions, children with autism had higher mean CBMN frequency of 12.73. Mothers of study subjects with family history of infertility / subfertility (12.04), family H/o cancer, family H/o chronic illness were observed with highest mean CBMN frequency. Mothers of the study subjects with late onset of menarche also had the highest mean CBMN frequency. In the current study subjects with normal karyotype showed a mean CBMN frequency of 11.7 and those with abnormal karyotype showed a mean CBMN frequency of 11.9.

Children born to women with PCOS, who belonged to low socioeconomic status showed a high mean CBMN frequency (12.2) followed by high (11.9) and medium (11.7) economic status. Duration of married life of women with PCOS was ranged from 3 to 11 years. Children born to mothers with highest duration of married life (9 to 11 years) showed a high mean CBMN frequency of (12.1). Study subjects with consanguineous parents showed a highest mean CBMN frequency.

Discussion

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder that is associated with multiple metabolic abnormalities (Goodzari et al, 2011). Among PCOS women, more than 60% manifest infertility (primary/secondary), and 19% experience amenorrhea (Balen et al, 1995). Moreover, pregnancy in PCOS women is more likely to be complicated by gestational diabetes, preeclampsia and preterm labor leading to miscarriage.

Obesity in PCOS further increases resistance to ovulation induction treatment since obesity is associated with a disturbed pattern of gonadotrophin releasing hormone production resulting in chronic elevation of tonic LH level with negative consequences on follicular development in the ovary (Norman et al, 2000). Besides these long term complications, infertility and poor pregnancy outcome are the major problems in PCOS patients (Zhang et al, 2014). However 60% of women with PCOS are fertile (defined as the ability to conceive within 12 months), although time to conceive is often increased (Brassard et al, 2008). In the present study it revealed that PCOS mothers with the family history of infertility / subfertility has higher mean CBMN frequency.

The imbalance between prooxidants and antioxidants can lead to a number of reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility (Agarwal et al, 2012). The present study showed that mothers with endometriosis had a highest mean CBMN frequency.

Cigarette smoking, alcohol use, and recreational drug use have been implicated in the pathogenesis of perturbed female reproductive mechanisms, leading to increased times to conception and infertility (Wells et al, 2005). These findings are in correlation with the present study, i.e.; usage of contraceptive drugs had a highest mean CBMN frequency (12.4).

According to Hardiman et al (2003), women with PCOS are also thought to be at increased risk for endometrial cancer through chronic anovulation with unopposed estrogen exposure of the endometrium. The present study revealed that the mothers with family h/o cancer had highest mean CBMN frequency.

According to Azziz et al (2004), problems with ovulation or menstruation are common in PCOS, and most women with this condition have menstrual irregularities. PCOS mothers with menstrual irregularities showed an increased mean CBMN frequency in the current study.

Vidal et al (2004) reported that higher incidence of chromosomal abnormalities was observed in a cohort of polycystic ovarian syndrome patients compared to normal patients. These increased rates of chromosomal abnormalities would be responsible of the high miscarriage rates observed in these patients. Children with normal karyotype were reported with the mean CBMN frequency 11.7 and that of those with abnormal karyotype was 11.9.

Unsaturated fatty acid peroxidation is a radical chain reaction and the products of lipid peroxidation reactions have been widely employed as biomarkers for oxidative stress. MDA, produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a good biomarker (Abuja and Albertini, 2001). This suggests that MDA value plays an important role in measuring the level of oxidative stress. The present study showed that the Malondialdehyde level was found to be raised in study subjects (1.21) and also found association with low socioeconomic status, increased duration of married life, infertility, habit of smoking, alcohol consumption, increased drug intake etc. The MDA value is comparable with that of the control subjects (0.93). The mean CBMN frequency with respect to the demographic and clinical risk factors showed increased level of somatic DNA damage in CBMN assay. The study demonstrated a positive correlation with PCOS and the extent of somatic DNA damages in subjects with more risk factor associated with genetically based conditions and demographic variables.

Conclusion

The present study observed the distribution of mean CBMN frequency and MDA value according to various demographical, clinical and lifestyle characteristics among the study subjects born to mothers with polycystic ovarian syndrome. These findings suggest that children born to women with PCOS have a high incidence of genomic instability. The present study can be concluded that hyperandrogenism, hyperinsulinemia and perhaps oxidative stress are factors contributing to increased micronuclei frequency and chromosomal damage. Thus oxidative stress can be considered as one of the important causes for abnormal endometrial environment with poor embryo receptivity in PCOS patients.

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