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EVIDENCE OF GENETIC INSTABILITIES AMONG WOMEN WITH POLYCYSTIC OVARIAN SYNDROME AND INFERTILITY

Suji Sundar LS Department of Zoology, SreeNarayana College, Cherthala- 688582, Kerala

PS Vidya Assistant Professor, Department of Zoology, SreeNarayana College, Cherthala-688582, Kerala

Dinesh Roy D Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram - 695024, Kerala

ABSTRACT

PCOS is one of the most common endocrinopathies. It occurs in 4-8% of women during their reproductive years. Infertility in PCOS patients was related to the effect of follicular fluid's oxidative stress levels on the meiotic spindle formation in the oocyte. The PCOS is a familial disorder, but the genetic basis of the syndrome remains controversial. Hence aim of the study was to evaluate the evidence of genetic instabilites among women with PCOS and infertility. The present study includes 45 study subjects and 25 healthy control subjects. The extent of somatic DNA damage was quantified by Cytokinesis-Block Micronuclei (CBMN) assay. Detailed demographic, clinical and lifestyle characteristics were compared with subjects. The micronuclei frequency was significantly elevated in study subjects than the control subjects. Various risk factors such as demographic, biochemical and lifestyle characters were showed increased micronuclei frequency which leads to infertility. Lifestyle modification is very important in the treatment for PCOS, as weight loss and exercise have been shown to lead to improved fertility and lowering of androgen levels. The high incidence of genetic instabilities in subjects with PCOS and infertility clearly warrants periodical examinations and counseling.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common metabolic dysfunction and heterogeneous endocrine disorder in women of reproductive age (Wood et al., 2007). PCOS is characterized by a clustering of hyperandrogenism, hyperinsulinemia, hyper secretion of LH, menstrual dysfunction, hirsutism, infertility, pregnancy and neonatal complications (Qiao and Feng, 2011).

A number of factors that is associated with PCOS (Rosenfield, 2007). Obesity is considered one of the most important features of PCOS (Glueck et al., 2001). Childhood obesity is a well-documented risk factor for PCOS. Obese girls are at a higher risk of developing insulin resistance, metabolic syndrome, and PCOS later on in life (Pasquali et al., 2011).

Infertility in PCOS patients was related to the effect of follicular fluid's oxidative stress levels on the meiotic spindle formation in the oocyte. It is generally admitted that PCOS is a multifactorial disorder that results from a combination of multiple gene polymorphisms under the influence of environmental factors (Barber and Franks, 2013). Infertility affects 40% of women with PCOS (Teede, Deeks and Moran, 2010). PCOS is the most common cause of anovulatory infertility. Women with PCOS have a normal number of primordial follicles and primary and secondary follicles are significantly increased. However, due to derangements in factors involved in normal follicular development, follicular growth becomes arrested as follicles reach a diameter of 4–8 mm. Because a dominant follicle does not develop, ovulation does not ensue (Teede, Deeks and Moran, 2010).

Genetic studies generally admitted that PCOS is a multifactorial disorder that results from a combination of multiple gene polymorphisms under the influence of environmental factors (Barber and Franks, 2013). Cytogenetic studies have shown that women with PCOS have increased damage in their genetic material (Moran et al., 2008; Yesilada et al., 2006).

The choice of treatment for women with PCOS depends on the symptoms with which a patient presents. Symptoms typically fit into three categories: menstruation related disorders, androgen related symptoms and infertility (Badawy and Elnashar, 2011). Treatment has been symptomatic including the infertile subgroup. However better understanding of the pathogenesis and more in depth knowledge of its genetic basis will make better treatment options available. Hence the present study was undertaken to study the evidence of genetic instabilities among women with PCOS and infertility.

Materials and Methods

45 subjects with PCOS and twenty five subjects without any chronic illness as control were selected for this study. Subjects were referred from various gynaecology and infertility centre of Kerala to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. Detailed demographic, clinical and lifestyle characteristics were recorded using proforma. In this study, Cytokinesis Block Micronuclei (CBMN) assay was carried out in each subject. CBMN assay was performed by using Cytochalasin B for quantitating the extent of somatic DNA damages. The fresh blood was collected by venipuncture and transferred to sodium heparin vacutainer. Added 5 to 6 drops of whole blood samples to a vial containing 10 mL RPMI 1640 medium supplemented with 100 units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 10µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5**µ**g/mL (Sigma) at 44th hour of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M 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 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

Observations and Results

The demographic and physiological findings of 45 study subjects were compared with 25 control subjects. The age of the study subjects ranged from 20 to 36 years with a mean age of 25.48 years and the age of the control subjects ranged from 18 to 35 years with a mean age of 27.48 years. 33 were belonged to rural area followed by urban (n=7) and coastal area (n=5).

Table 1: Distribution of mean CBMN frequency according to study and control subjects

Variables	Number	Mean CBMN frequency
Study subjects	45	12.43
Control subjects	25	10.83

Distribution of mean CBMN frequency according to demographic characters

Table 2 showed thatincreasing age and birth order of subjects were showed highest mean CBMN frequency. Majority of the subjects were residing in rural area but subjects residing in urban area were showed highest mean CBMN frequency of 12.86. Most

of the subjects were Hindus, Christians having highest mean CBMN frequency. Subjects with sedentary type of work were showed highest mean CBMN frequency. Subjects with high socioeconomic subjects were showed high mean CBMN frequency than low and average socio economic subjects. Subjects were grouped BMI into <25, 25 to 30 and >30 kg/m^2. Subjects with obese and overweight subjects were showed highest mean CBMN frequency of 12.48 and 13.02. 3 subjects having consanguineous marriage and they showed 13.28 as their mean CBMN frequency. 6 subjects with parental consanguinity and they showed 12.77 as mean CBMN frequency.

Distribution of mean CBMN frequency according to clinical characters

In table 3, subjects with family H/o infertility were showed highest mean CBMN frequency of 12.59. 5 subjects with family H/o PCOS and their mean CBMN frequency was 13.19. Age at menarche of the subjects were grouped into 10 to 15 and >15 years. As the age at menarche increases mean CBMN frequency of thesubjects also increases. Subjects with irregular menstruation were showed mean CBMN frequency of 12.54.8 subjects were showed endometriosis and they have 13.15 as mean CBMN frequency. 13 subjects with use of contraceptive drugs used, these have highest mean CBMN frequency.

Distribution of mean CBMN frequency according to biochemical and hormonal

Biochemical and hormonal characters of the subjects were shown in table 4. Subjects with FBS above normal range were showed highest mean CBMN frequency of 12.58. Subjects with >200 mg/dl of total cholesterol were showed highest mean CBMN frequency. Subjects having abnormal level of HDL and LDL cholesterol were showed highest mean CBMN frequency. Subjects with >150 mg/dl of triglyceride were showed mean CBMN frequency of 12.46. Subjects with abnormal level in hormones were showed highest mean CBMN frequency.

Discussion

According to Moran et al., (2008) DNA of anovulatory infertile subjects showed significant damage, by increased mean CBMN frequency in lymphocytes. The studies showed the presence of genetic abnormality in PCOS subjects (Yesilada et al., 2006). Current study also showed genetic instabilities higher in PCOS subjects than controls.

In the present study showed increased mean CBMN frequencies with advancing age were consistent with the results of international collaborative projects (Bonassi etal., 2003). In the current study it was observed that micronuclei frequency (MN) was highest in the age group>30 years, there seems to be relation between age and micronuclei frequency.

Majority of the study subjects were belonged to rural area (70.83%) followed by coastal area (16.67%) and urban area (12.5%) and the highest mean CBMN frequency was observed in urban area (13.87) (Arun et al., 2016). The present study also showed the highest mean CBMN frequency (12.86) shown by subject's belonged to urban area.

According to Li et al., (2011) those subjects, with high economic status showed highest mean CBMN frequency. In the present study, subjects with high economic status showed high mean CBMN frequency.The increase in genomic damage in obese subjects and the positive correlation between genomic damage and BMI in total over-weight/obese subjects indicate that obesity increases genomic damage (Donmez-Altuntas, 2014). According to Teede et al., (2013) BMI is higher in women with PCOS and is a known independent risk factor for infertility (Kumbak, Oral and Bukulmez, 2012). The present study showed BMI >30 kg/m^2 have highest mean CBMN frequency of 13.02.

Dyslipidaemia is very common in PCOS patients (up to 70% alone in the United States) (Essah et al., 2008). Many studies have exposed that LDL-C is increased in women with PCOS. The present study also observed that LDL cholesterol values were higher among study subjects than the normal control subjects.

The present study shows that the overall prevalence of elevated level of fasting blood sugar in women with PCOS is 77.78% (n=35). In comparison, a study done by Pasquali et al., (2011) reported prevalence of 8.3% had impaired fasting glucose. A high glucose level can point to insulin resistance, a diabetes related ailment that contributes to PCOS.

According to Balen, (2007) the endocrine abnormalities in women with polycystic ovary syndrome include raised concentrations of luteinising hormone (LH; seen in about 40% of women), testosterone and androstenedione in association with low or normal concentrations of follicle stimulating hormone. The study observed an increased FSH value and increased LH value. All these characteristics showed a significant increase with mean CBMN frequency among the anovulatory subjects. The serum levels of FSH, LH and prolactin were significantly increased in the study subjects of the present study.

Conclusions

The findings of the present study imply that there is a strong evidence of genetic instability. Risk factors of subjects were showed increased susceptibility to DNA damage. Lifestyle modification is very important in the treatment for PCOS, as weight loss and exercise have been shown to lead to improved fertility and lowering of androgen levels. Dietary weight loss is recommended as the primary treatment strategy. It also reduces the long term risk of diabetes, CVD and possibly endometrial cancer. The high incidence of genetic instabilities in subjects with PCOS and infertility clearly warrants periodical examinations and counseling.

TABLE 2 :

Variables	Category	Number	Percentage (%)	Mean CBMN Frequency
Family H/o infertility	No	42	93.33	12.47
	Yes	3	6.66	12.59
Family H/o PCOS	No	40	88.88	12.34
	Yes	5	11.11	13.19
H/o X -ray exposure	No	42	93.33	12.42
	Yes	3	6.66	12.68
Age at menarche (Year)	10 to 15	37	82.22	12.4
	>15	8	17.77	12.59
Menstrual periods	Irregular	30	66.66	12.54
	Regular	15	33.33	12.23
Endometriosis	No	37	82.22	12.28
	Yes	8	17.77	13.15
Contraceptive drugs used	No	32	71.11	12.26
	Yes	13	28.88	12.86

TABLE 3 :

Variables	Category	Number	Percentage (%)	Mean CBMN Frequency
Age (Year)	<30	41	91.11	12.41
	≥30	4	8.88	12.63
Birth order	1 to 5	40	88.88	12.41
	>5	5	11.11	12.66
Residence	Rural	33	73.33	12.33
	Urban	7	15.55	12.86
	Coastal	5	11.11	12.54
Religion	Hindu	35	77.77	12.38
	Muslim	4	8.88	12.32
	Christian	6	13.33	12.83
Education	Graduate/PG	16	35.55	12.87
	Higher secondary	28	62.22	12.21
	Primary	1	2.22	11.65
Occupation	Non sedentary	29	64.44	12.35

	Sedentary	16	35.55	12.58
Economic status	High	4	8.88	12.79
	Medium	40	88.88	12.42
	Low	1	2.22	11.65
BMI (Kg/m^2)	<25	16	35.55	11.99
	25 -30	18	40	12.48
	>30	11	24.44	13.02
Consanguinity	No	42	93.33	12.37
	Yes	3	6.66	13.28
Parental consanguinity	No	39	86.66	12.38
	Yes	6	13.33	12.77

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