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Genetics

BIOCHEMICAL AND GENETIC STUDIES ON POLYCYSTIC OVARIAN SYNDROME AND ITS RISK ON CARDIOMETABOLIC SYNDROME

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ABSTRACT Polycystic Ovarian Syndrome is the major cause of anovulatory infertility and a common genetical endocrinopathy with a variety of clinical symptoms in women of reproductive age. It is characterized by anovulation, hyperandrogenism and polycystic ovarian morphology. A study was conducted to evaluate the biochemical and genetic studies on PCOS and its risk on cardiometabolic syndrome. There were 71 study subjects in the age group of 20 to 36 years and 33 age matched control subjects. Cytokinesis block micronuclei assay (CBMN) was carried out to measure the extent of somatic DNA damages. Serum lipid profile and hormonal analysis were also estimated in this era of all the subjects after obtained their informed consent. Obtained results were made to correlate with cardiometabolic risk. Decreased HDL and elevated levels of all other parameters were observed in the study subjects. A significantly elevated level of micronuclei (MN) in the study subjects along with the above parameters and body mass index (BMI) is increased risk of CVD in PCOS.

KEYWORDS : Polycystic Ovarian Syndrome, Hormonal imbalance, Cardiometabolic syndrome and Cytokinesis block micronuclei assay

INTRODUCTION:

Polycystic Ovarian Syndrome (PCOS) also called hyperandrogenic anovulation, or Stein-Leventhal syndrome (Kollman et al., 2014). PCOS is a common reproductive disorder, defined by two out of three criteria: (1) Menstrual irregularity (2) Hyperandrogenism and (3) Polycystic ovarian morphology (Rotterdam, 2004).

PCOS affects 7–10% of women worldwide. The overall prevalence was reported to be 6 to 8 in the general population (Azziz et al., 2004). The risk factors for the onset of PCOS include obesity, lack of physical exercise, family history and diabetes mellitus. The hormonal factors include GnRH, LH/FSH ratio, estrogens, androgens, insulin, cortisol, growth hormone, vitamin D, PTH (parathyroid hormone) and calcitonin (Kandarakis and Legro, 2006).

Pathogenesis of PCOS is still unclear but is thought to be multifactorial, consisting of endocrine, metabolic, genetic and environmental factors. Genetic and environmental contributors to hormone disturbances combine with other factors, including obesity, ovarian dysfunction and hypothalamic pituitary abnormalities to contribute to the aetiology of PCOS (Coviello et al., 2006). A lot of investigations have revealed that oxidative stress level is significantly increased in patients with PCOS. Reactive oxygen species could cause genetic changes and leading to DNA damages (Ziech et al., 2011).

PCOS women with metabolic syndrome and/or type 2 diabetes are in the high risk group for CVD (Wild et al., 2010). Infertility is also a very common and troubling issue facing married women with PCOS and is at great risk for mental health problems, such as anxiety and depression. Defect in reproductive health of a human will cause future health problems and it also predicts future CVD diseases. Hence the present study was undertaken to quantify the extent of DNA damage by Cytokinesis Block Micronuclei (CBMN) assay along with evaluating the biochemical parameters in PCOS subjects and to assess the risk for CVD.

MATERIALS AND METHOD:

Seventy one women with PCOS and thirty three healthy age matched women were selected for the study. The samples were recruited from various maternity centers of Kerala for genetic testing to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala. Detailed demographic, lifestyle and clinical characteristics were recorded using proforma. Cytokinesis-Block Micronuclei (CBMN) Assay was performed on each sample by using Cytochalasin B for quantitating the extent of somatic DNA damages.

Eight ml of venous blood was collected aseptically from all the subjects by venipuncture after overnight fasting. 4ml was transferred into the sodium heparin vacutainer to perform CBMN assay. The remaining 4ml was transferred into plain tube and allowed to clot. With the serum, sugar and lipid profile were estimated by enzymatic method using MISP A neo semi automated clinical chemistry analyzer. Lutinizing hormone (LH), Follicle stimulating hormone (FSH), and Estradiol were measured by the Chemi Luminescent Immuno Assay (CLIA) using Beckman Access 2 fully automated hormone analyzer.

Lymphocyte cultures were prepared for each subject and were performed in 10 ml RPMI 1640 supplemented with 100 units/ ml penicillin, 100 units/ml streptomycin, 15% foetal bovine serum and 10µg/ml phytohemagglutinin. At 44th hr after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, final concentration, 4.5µg/ml). Cells were harvested after 72th hr incubation, and they were treated with a hypotonic KCl 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:

In the present study, 71 study subjects showed a mean CBMN frequency of 12.92 while the control subjects were showed mean CBMN frequency of 10.27. This difference in mean CBMN frequencies showed a statistically significant difference.

TABLE 1:- DISTRIBUTION OF MEAN CBMN FREQUENCY ACCORDING TO VARIOUS DEMOGRAPHIC CHARACTERISTICS

Category	Variables	Number (Percentage)	Mean CBMN frequency
Age (Years)	≤25	33 (46.47%)	12.8
	26-30	23 (32.39%)	12.91
	31-36	15 (21.12%)	12.99
Birth order	≤3	53 (74.64)	12.9

	>3	18 (25.35%)	12.93
Residence	Rural	45 (63.38%)	12.82
	Urban	15 (21.12%)	12.92
	Coastal	11 (15.49%)	13.1
Occupation	Sedentary	18 (25.35%)	13.04
	Non Sedentary	53 (74.64%)	12.89
Menstrual periods	Regular	26 (36.61%)	12.88
	Irregular	45 (63.38%)	13.01
BMI	<25	29 (40.84%)	12.81
	≥25	42 (59.15%)	13
Parental consanguinity	Yes	4 (5.63%)	13.05
	No	67 (94.36%)	12.92
Economic Status	Low	3 (4.22%)	13.09
	Medium	61 (85.91%)	12.92
	High	7 (9.85%)	12.85

The subjects were grouped on their demographic characteristics (Table 1). The highest mean CBMN frequency of 12.99 was shown by 15 subjects

between the age of 31 to 36 years. The highest mean CBMN frequency (12.93) shown by subjects having >3 birth order. Majority of the study subjects were belonged to rural (63.38%) area and highest mean CBMN frequency observed with coastal residing. Subjects having sedentary type of work were showed highest mean CBMN frequency of 13.04. Majority of study subjects with irregular menstrual period and they had highest mean CBMN frequency. Obese subjects have highest micronuclei frequency. Subjects with parental consanguinity showed highest mean CBMN frequency of 13.05. 3 (4.22%) subjects were showed a low economic status with a highest mean CBMN frequency of 13.09.

TABLE 2:- DISTRIBUTION OF MEAN CBMN FREQUENCY ACCORDING TO CLINICAL FINDINGS OF THE STUDY SUBJECTS

Category	Variables	Number (Percentage)	Mean CBMN frequency
Family H/o PCOS	Yes	5 (7.04%)	13.73
	No	66 (92.95%)	12.86
Family h/o infertility/subfertility	Yes	4 (5.63%)	13.37
	No	67 (94.36%)	12.9
Family h/o cancer	Yes	2 (2.81%)	13.3
	No	69 (97.18)	12.92
H/o Chronic illness	Yes	5 (7.04%)	13.28
	No	66 (92.95%)	12.9
Family H/o CAD	Yes	17 (23.94%)	13.42
	No	54 (77.14%)	12.77
H/o Diabetes Mellitus	Yes	22 (30.98%)	13.32
	No	49 (69.01%)	12.75
Age at menarche (Years)	≤14	53 (74.64%)	12.85
	>14	18 (25.35%)	13.16

Among 71 study subjects, 5 subjects (7.04%) have family H/o PCOS. These subjects have high value of mean CBMN frequency of 13.73. Only 4 subjects (5.63%) were having family h/o infertility/ subfertility showed high mean CBMN frequency of 13.37. 2 subjects (2.81%) with family h/o cancer among first and second degree of relatives showed a highest mean CBMN frequency of 13.3. 5 subjects (7.04%) have h/o chronic illness with mean CBMN frequency of 13.28. The mean CBMN frequency was higher in those which had the h/o chronic illness. 17 subjects (23.94%) were showed family h/o CAD and they have highest mean CBMN frequency of 13.42. 22 subjects (30.98%) have H/o diabetes mellitus and they have highest mean CBMN frequency of 13.32.

The highest mean CBMN frequency (13.16) was reported in subjects with age at menarche >14 years of age.

TABLE 3:- COMPARISON OF BIOCHEMICAL AND ENDOCRINOLOGICAL CHARACTERISTICS OF STUDY SUBJECTS

Category	Variables	Number (Percentage)	Mean CBMN frequency
FBS (mg/dL)	≤110	12 (16.90%)	12.78
	>110	59 (83.09%)	12.95
Total Cholesterol (mg/dL)	≤200	25 (35.21%)	12.79
	>200	46 (64.78%)	13
HDL Cholesterol (mg/dL)	<50	64 (90.14%)	12.94
	≥50	7 (9.85%)	12.75
LDL Cholesterol (mg/dL)	≤100	5 (7.04%)	12.75
	>100	66 (92.95%)	12.94
TG (mg/dL)	<150	51 (71.83%)	12.89
	≥150	20 (28.16%)	13.02
FSH (m IU/mL)	≤30	53 (74.64%)	12.88
	>30	18 (25.35%)	13.05
LH (mIU/mL)	<30	6 (8.45%)	12.22
	≥30	65 (91.54%)	12.99
Estradiol (pg/mL)	≤75	38 (53.52%)	12.92
	>75	33 (46.47%)	12.93

The subjects were grouped on their various biochemical and endocrinological characteristics. 59 subjects have FBS level of >110 mg/dL with high mean CBMN frequency of 12.95. 46 subjects have total cholesterol of >200 mg/dL with high mean CBMN frequency of 13. In case of HDL cholesterol, 64 subjects have highest mean CBMN frequency with <50 mg/dL of HDL. Subjects with LDL cholesterol (92.95%) in the range of >100 mg/dL have highest mean CBMN frequency of 12.94. In case of triglyceride level ≥150 mg/dL have the mean CBMN frequency of 13.02. According to endocrinological characters of the subjects the highest mean CBMN frequency shown by subjects with abnormal hormone levels.

DISCUSSION:

The present study shows that the women with PCOS have the family history of PCOS. A previous study by Azziz and Kashar-Miller, (2000) noted that 35% of mothers and 40% of sisters of patients with PCOS

will be affected by PCOS themselves, which is consistent with the results of this study.

Balen et al., (1995) reported a high prevalence of family history of primary and secondary infertility in a large group of PCOS patients. In addition, a higher incidence of chromosomal instability in the infertile population is widely recognized (Trkova et al., 2000). Similarly this study clearly indicates that there is an increase in DNA damage in patients with the family history of primary and secondary infertility.

Orio et al., (2005) have shown the detrimental effect of PCOS on the cardiovascular system, even in young women asymptomatic for cardiac disease. As same as that this study reported that PCOS is associated with an increased risk of CVD.

Mokhtar et al., (2006) revealed that females with the age of menarche more than 15 years were more risky to develop infertility than those with age of menarche less than 15 years. In the present study, the subjects with advancing age of menarche were showed a high mean CBMN frequency.

A study by Suhail et al., (2008) suggested that there were no increase in TC and LDL-C levels but decreased HDL-C levels in subjects with PCOS without increased triglyceride levels. In the present study elevated levels of TC, LDL and TG and decreased levels of HDL was observed. This is suggestive of an increased risk for CVD in patients with PCOS.

Lavanya et al., (2008) reported that only small population of PCOD patients had elevated LH levels. Johanna et al., (2011) demonstrated that FSH levels are lower in infertile PCOS women. According to the present study majority of PCOD patients showed elevated LH levels. Subjects with elevated LH level show highest mean CBMN frequency. The mean FSH level in the study subjects was significantly higher than

that of the controls with mean CBMN frequency of 13.05.

CONCLUSION:

The present study involves biochemical and genetic studies on PCOS and its risk on cardiometabolic syndrome. The distribution of mean CBMN frequency according to demographic, clinical, and biochemical characteristics of the study subjects was observed. Age, birth order, parental consanguinity and BMI etc were showed increased level of mean CBMN frequency. The level of mean CBMN frequency high among those have the family history of PCOS, infertility/subfertility, cancer and the history of chronic illness, coronary artery diseases, and diabetes mellitus. FBS, total cholesterol, FSH, LH and Estradiol were found to be significantly elevated in study subjects. These findings suggest that the women with PCOS have high risk for cardiometabolic syndrome. While PCOS cannot be prevented or cured, it can be controlled, with varying degrees of success, by maintain a healthy diet and by exercising. Healthy lifestyle factors, including exercise, are associated significantly with reduced DNA damage.

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