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RESEARCH ARTICLE

OXIDATIVE STRESS AND GENETIC INSTABILITIES IN POLYCYSTIC OVARIAN SYNDROME.

Aneesh P[1], AP Krishna[2], E Kayalvizhi[3], Drisya K[4], Anaswara AA[4] and Dinesh Roy D[4]

1. Research Scholar, Meenakshi University, West K K Nagar, Chennai, Tamil Nadu, India.
2. Department of physiology, K S Hegde Medical Academy, Mangalore, Karnataka, India.
3. Department of physiology, Meenakshi Medical College, Research Institute, Kanchipuram, Tamil Nadu, India.
4. Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram - 695024, Kerala.

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Abstract

The polycystic ovary syndrome (PCOS) is a hyper androgenic disorder associated with chronic oligo-anovulation and polycystic ovarian morphology. Women with PCOS have an increased risk of miscarriage, gestational diabetes, preeclampsia and preterm labour. The pathogenesis of PCOS is however unclear but is thought to be multifactorial consisting of endocrine, metabolic, genetic and environmental factors. The present study consists of 40 study subjects with PCOS and 35 healthy control subjects. Detailed demographic, clinical and lifestyle characteristics were recorded and compared with other clinical parameters. The association of various physiological and lifestyle factors which leads to oxidative stress was analysed by evaluating MDA concentration and subsequent DNA damages, if any, was quantified by Cytokinesis-block Micronuclei (CBMN) assay. Study subjects demonstrated a statistically significant increased serum MDA level and mean CBMN frequency than the control subjects. Subjects with abnormal biochemical, physiological and endocrinological characters showed increased mean CBMN frequency. These findings denoted that there is a strong evidence of genetic instability among subjects with PCOS. Hence the study can be concluded that, oxidative stress and somatic DNA damages may play a major role in the risk of PCOS and further complications. Optimal metabolic control and lifestyle modification can reduce the unwanted clinical symptoms of infertility. Healthy lifestyle factors, including exercise, are associated significantly with reduced DNA damages.

Introduction:-

The polycystic ovary syndrome (PCOS) is a hyperandrogenic disorder associated with chronic oligo-anovulation and polycystic ovarian morphology (Azziz et al., 2006). Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting approximately 5–10% of women in reproductive age with associationbetween amenorrhea, hirsutism, obesity and polycystic ovaries (Stein et al., 1935). The PCOS affects 7 to 8% of women (Azziz et al., 2004) and may be the most common cause of female infertility (Norman et al., 2002). Women with PCOS have an increased risk of miscarriage, gestational diabetes, preeclampsia and preterm labour

Corresponding Author:-Dinesh Roy D.
Address**:-**Genetika, Centre for Advanced Genetic Studies, Pettah P O, Trivandrum - 695024, India.

(Boomsma et al, 2006). PCOS is the most common cause of menstrual irregularity that leads to infertility. In young women with PCOS, multiple risk factors for Cardiovascular Disease (CVD), including metabolic syndrome (MBS), type 2 diabetes mellitus (T2DM), dyslipidemia, abdominal obesity and hypertension may be found. Increased adiposity in PCOS women interact to promote premature atherosclerosis and increase cardiovascular mortality is a worldwide concern (Wild et al., 2010).

The close association between oxidative stress and lifestyle-related diseases has become well known. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins and DNA. Lipid degradation occurs, forming products such as malondialdehyde (MDA) and ethane that are commonly measured as end products of lipid peroxidation. Human DNA remains continuously in exposure to free radicals attack. The majority of DNA damage occurs in human beings in response to oxidative stress (OS). The pathogenesis of PCOS is however unclear but is thought to be multifactorial consisting of endocrine, metabolic, genetic and environmental factors. Even though root cause behind PCOS is unknown, further research is needed to evaluate the predisposing factors, particularly genetic background and environmental factors such as endocrine disruptors and lifestyle that increases the risk of PCOS. Hence the present study was undertaken to evaluate the association of various physiological and lifestyle factors which leads to oxidative stress and subsequent DNA damages in women with PCOS.

Materials and Methods:-

The study subjects comprised of forty women in the age group of 20 to 36 years with a clinical diagnosis of PCOS referred from various gynecology and infertility centers of Kerala to Genetika, Centre for advanced Genetic studies, Trivandrum. Twenty healthy study subjects having regular menstruation and without any chronic illness were selected as control for this study. Various demographical, physiological, life style, clinical and biochemical characteristics of the subjects were analysed. Venous blood was collected aseptically from all the subjects. 5 ml of venous blood was collected from all the subjects by venipuncture. From that, 2 ml blood was transferred to a sterile vacuutainer and was used for CBMN assay and remaining 3 ml blood was transferred in to a plain tube and allowed to clot. The biochemical parameters such as Fasting Blood Sugar, Total cholesterol, Triglyceride, HDL-C and LDL-C were estimated by enzymatic method. Hormones viz. Luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin and estradiol were also performed. MDA was performed by TBA method to evaluate the oxidative stress. Cytokinesis-block Micronuclei Assay (CBMN) assay was performed by using Cytochalasin-B for quantitating the extent of somatic DNA damages.

Observations and Results:-

The demographic and physiological findings of 40 study subjects were compared with 35 control subjects. The age of the study subjects ranged from 20 to 36 years with a mean age of 28.2 years and age of control subjects ranged from 17 to 35 years with mean age of 27.05 years. Family history of PCOS was reported in 8 out of 40 study subjects. Family history of infertility/sub-fertility was reported in 3 study subjects. The history of chronic illness was reported among 2 out of 40 study subjects. Majority of the study subjects (n=38) attained menarche on or before 16 years of age and the remaining two have menarche at the age above 16 years. Out of the 40 study subjects, irregular menstruation was reported in 9 subjects. Five study subjects had endometriosis. The study subjects showed a statistically significant increase in MDA level than the control subjects ($t=5.477$; $p=<.00001$) The CBMN analysis revealed that, the study subjects showed a mean CBMN frequency of 13.18 ± 0.783 and control subjects showed a mean CBMN frequency of 10.88 ± 0.384 ($t=13.032$; $p=<.00001$).

The study revealed that, the mean CBMN frequency increases with increased age. Among the 40 study subjects, age between 32-37 years showed highest mean CBMN frequency (13.31). Study subjects belonged to birth order >5 showed an increased mean CBMN frequency (13.3). Based on residence of these study subjects, highest mean CBMN frequency was observed among subjects who belonged to coastal area (13.34). Moreover, increased mean CBMN frequency was observed among subjects with sedentary type of occupation and subjects with higher level of socio-economic status.

Significantly increased mean CBMN frequency was observed in subjects with family history of (FH/o) PCOS compared to that of subjects without FH/o PCOS. Similarly, increased mean CBMN frequency was observed in subjects with history of (H/o) chronic illness. Study subjects with the FH/o infertility/sub-fertility showed a high

mean CBMN frequency of 13.41. The mean CBMN frequency of subjects with endometriosis was higher than that of the subjects without endometriosis.

Among the study subjects, 31 subjects reported regular menstrual periods and 9 reported irregular menstrual periods. Subjects with irregular menstrual periods showed increased MDA level than the subjects with regular menstrual periods. CBMN analysis revealed that subjects with irregular menstrual periods also showed an increased mean CBMN frequency than the rest. The subjects who had menarche at age >16 years had a higher mean CBMN frequency (13.19) than subjects with menarche at the age ≤16 years. Moreover, subjects with these risk factors showed increased MDA level than the subjects without these risk factors. It is also true that, a positive correlation was observed between mean CBMN frequency and MDA level. Fourteen out of forty subjects reported obesity and these subjects showed increased mean CBMN frequency and MDA level (Table: 1).

Table 1:-Distribution of mean CBMN frequency and MDA concentrations according to clinical and physiological characteristics:-

Category	Variable	Number	MDA	Mean CBMN Frequency
Family History of (FH/o) PCOS	Yes	8	2.56	13.36
	No	32	2.40	13.13
History of (H/o) chronic illness	Yes	2	2.59	13.35
	No	38	2.44	13.17
FH/o infertility and sub-fertility	Yes	3	2.41	13.41
	No	37	2.56	13.16
Endometriosis	Yes	5	2.77	13.85
	No	35	2.39	13.22
Menstrual periods	Regular	21	2.08	13.16
	Irregular	9	2.73	13.56
Menarche	≤16	38	2.12	13.03
	>16	2	2.6	13.19
Obesity	Yes	14	2.55	13.2
	No	26	2.01	12.81

The study frankly demonstrated that, the mean CBMN frequency was higher among subjects who had increased FBS, Total Cholesterol, Triglyceride and LDL-C. While, subjects had low level of HDL-C showed increased mean CBMN frequency (Table: 2). Hormonal analysis revealed that, subjects with increased FSH and LH showed higher mean CBMN frequency. Moreover, subjects with increased FBS, Total Cholesterol, Triglyceride and LDL-C and decreased HDL-C level showed increased MDA values. Increased MDA level was also observed among subjects with, increased FSH and LH levels.

Table 2:-Distribution of mean CBMN frequency and MDA concentrations according to biochemical parameters:-

Category	Variables	Number	MDA (U/L)	Mean CBMN frequency
FBS (mg/dL)	≤ 110	10	2.3	12.23
	>110	30	2.86	13.2
TC (mg/dL)	≤200	16	2.39	13.06
	>200	24	2.56	13.26
TG (mg/dL)	≤150	26	2.32	13.12
	>150	14	2.79	13.28
HDL-C (mg/dL)	≤40	32	2.50	13.86
	>40	8	2.41	12.25

LDL-C (mg/dL)	≤100	3	2.37	13.14
	>100	37	2.83	13.27
FSH (mlU/ml)	≤30	29	2.35	13.17
	>30	11	2.79	13.21
LH (mlU/ ml)	≥30	3	2.3	12.82
	<30	37	2.86	13.21

Discussion:-

Polycystic ovary syndrome (PCOS) is the most common cause of female infertility due to anovulation and it affects approximately 6–10% of reproductive-aged women (Baillargeon et al., 2008). PCOD is a complex condition characterized by elevated androgen levels, menstrual irregularities and/or small cysts on one or both ovaries (Umland EM et al., 2011).

Kuscu et al., 2009 compared serum MDA level in PCOS patients with healthy controls. They showed the MDA concentrations were significantly higher in the PCOS patients. The present study also observed a statistically significant increased serum MDA level in subjects with PCOS.

Murri et al., 2013 reported increased MDA level in PCOS subjects and also reported that, circulating mean MDA concentrations according to the age and BMI were increased 47% in women with PCOS compared with controls. The present study also observed that, subjects who are obese showed increased MDA concentrations.

Patients with PCOS have higher gonadotropin releasing hormone (GnRH), which in turn results in an increase in LH/FSH ratio in females with PCOS. The majority of patients with PCOS have insulin resistance and/or obesity (Kabel, 2016). The present study observed that, among 40 study subjects, 29 subjects showed increased FSH level and 3 subjects showed increased LH level. Moreover, mean CBMN frequency was observed in subjects with elevated level of LH and FSH.

According to Josey et al., (2017) the highest mean CBMN frequency of 13.38 was shown by 35 subjects (46.6%) of age between 29 to 36 years. According to Tehrani et al., (2015) PCOS is a problem with hormones that affects women during their childbearing years (ages 15 to 44). The present study is in agreement with the above mentioned studies. Moreover, the present study also revealed that, the mean CBMN frequency was increased with increase in age.

In a study done by Arun et al., (2016) observed that, a relationship between birth order and extent of somatic DNA damages in PCOS and reported that the highest mean CBMN frequency (13.8) was demonstrated by subjects with birth order >6. According to Josey et al., (2017) subjects with increased birth order showed the increased mean CBMN frequency (13.7). Similarly, in this study also observed that, mean CBMN frequency was increased with increase in birth order. The highest mean CBMN frequency (13.3) was showed by subjects with >6 birth order.

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 this present study the subjects who had delayed menarche showed increased mean CBMN frequency.

Conclusions:-

In short, majority of the PCOS subjects showed abnormal biochemical, physiological and hormonal parameters. The study observed a statistically significant increased serum MDA concentration and mean CBMN frequency. Moreover, subjects with abnormal biochemical, physiological and endocrinological characters showed increased MDA concentration and mean CBMN frequency. There is a positive correlation between these risk factors with MDA concentration and mean CBMN frequency. The findings imply that there is a strong evidence of genetic instability. DNA damage may play a major role in the risk of PCOS and further complications especially, Cardiovascular risks. The present study highlights the molecular and physiological association of oxidative stress among women with PCOS.

This study proved significant evidence of oxidative stress that might play a crucial role in the pathogenesis of PCOS and hence, oxidative stress parameters could be suggested as diagnostic markers for early diagnosis of high-risk groups. Lifestyle modifications and proper dietary management to decrease the overweight and obesity would be able to reduce the unwanted clinical symptoms and hirsutism in females with polycystic ovary syndrome. Maintaining healthy lifestyle factors, regular exercise, good dietary pattern were also help to reduce DNA damages.

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