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Association of DNA damage and dyslipidemia with polycystic ovarian syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is associated with hyperinsulinemia and insulin resistance which may lead to cardiovascular diseases. Evidence for cardiovascular events in women who were affected by PCOS during fertile age is limited. The pathogenesis is unknown; however, it is a complex multigenetic disorder. The present study was undertaken to evaluate the various cardiovascular risk factors and their DNA repair efficiency in women with PCOS by investigating the biochemical, endocrinological and molecular cytogenetic alterations. These investigations were carried out in 116 women in the age group of 15-35 years clinically diagnosed with PCOS. Data were compared with that of 50 age-matched healthy normal women. Fasting blood sugar (FBS), Lipid profile, Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), Prolactin and Estradiol were estimated after getting the informed consent. Mutagen induced chromosome sensitivity analysis was carried out in the lymphocytes of the subjects to assess the DNA repair proficiency. Fasting Blood Sugar, total cholesterol and LDL cholesterol were found to be elevated whereas HDL cholesterol was found to be lowered in the test subjects. FSH, LH and prolactin were also found to be significantly elevated in the test subjects. Change in the estradiol concentration in the test subjects was not significant. The mutagen sensitivity analysis revealed a significant elevation in break per cell (b/c) values indicating a deficiency in the DNA repair mechanism / DNA damage in PCOS patients. Modification of life style by changing the dietary habit and sedentary life style will help to reduce the oxidative stress and may increase the ovarian function and a sensible lifestyle management is recommended for reducing the risk for CVD.

Key words: Polycystic ovary syndrome, cardiovascular disease, metabolic syndrome, type 2 diabetes mellitus, dyslipidemia, DNA repair mechanism

Polycystic ovary syndrome (PCOS) is a common condition estimated to affect 4-18% of women of reproductive age[1]. PCOS is associated with reproductive (hyperandrogenism, menstrual irregularity, anovulation, infertility and increased pregnancy complications), psychological (impaired quality of life and increased anxiety and depression), metabolic (increased risk factors for impaired glucose tolerance and type 2 diabetes mellitus) and cardiovascular disease sequelae[2].

PCOS is a spectrum of disorders with any combination of oligo/anovulation, clinical and/or biochemical evidence of androgen excess, obesity, insulin resistance and polycystic ovaries on ultrasound. The pathogenesis is unknown; however, it is a complex multigenetic disorder where decreased gonadotropin release, dysregulation of steroidogenesis, hyperinsulinism and insulin resistance plays a role[3].

Recent studies have shown that PCOS is associated with hyperinsulinemia and insulin resistance and may lead to cardiovascular diseases. Although evidence for cardiovascular events in women who were affected by PCOS during fertile age is limited, available data suggest more frequent cardiovascular disease (CVD) in classic PCOS. 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. With increased adiposity in two thirds of American PCOS women, the degree to which obesity and PCOS interact to promote premature atherosclerosis and increase cardiovascular mortality is a worldwide concern. There is increasing evidence indicating an elevated prevalence of impaired glucose tolerance (IGT), T2DM, MS and CVD in PCOS. CVD and T2DM are leading causes of morbidity and mortality in the Western society accounting for a great proportion of health care costs[4]. These diseases share common risk factors, including obesity, insulin resistance, elevated blood glucose, lipid oxidation toxicity and low-grade inflammation. They coexist in a great number of patients.

The existence of chromosomal aneuploidy in multinucleated endothelial cells may be important in atherogenesis by strongly expressing low density lipoproteins (LDL) receptors and increasing LDL uptake to the subendothelial intima. Chromosomal aberrations can also occur in vascular smooth muscle cells of human atherosclerotic plaques, especially in unstable plaques. DNA strand breaks, altered purines and oxidized pyrimidines are also significantly higher in leukocytes of patients with CAD, and DNA strand breaks levels are higher in patients with acute coronary syndrome as compared with patients with stable CAD[5].Various studies demonstrated the presence of chromosomal damage in circulating cells of patients with CAD using the Cytokinesis-block micronuclei (CBMN) assay[6-8]. In these studies, an elevated frequency of micronuclei (MNs) was significantly correlated with both the occurrence and the severity of CVD. Overall, these studies indicated that an increased MNs frequency is correlated to the pathogenesis of metabolic disorders and CVD risks.

There is now a greater focus on the management of the metabolic consequences of PCOS, primarily through lifestyle intervention to achieve weight loss and increase physical activity[9]. There are only few studies concerning the correlations between phenotypic expression, body composition and PCOS, and relationship with the processes of growth and sexual maturation and various environmental factors (nutrition, physical activity, stress, and other factors). No systematic studies were reported regarding the genetic integrity, especially the DNA damage in PCOS and the DNA repair efficiency. Hence, the present study was undertaken to evaluate the various CVD risk factors and their DNA repair efficiency in women with PCOS by investigating the hormonal, biochemical and molecular cytogenetic alterations.

Materials and Methods

One hundred and sixteen women in the age group of 15-35 years with a clinical diagnosis of PCOS referred from RBM Hospital, Alappuzha, Kerala formed the test group. Fifty age-matched healthy women formed the control group. Informed consent were obtained from all the subjects and detailed clinical and anthropometric characteristics were recorded using proforma. Pregnant and lactating women as well as diabetic patients were not included in the study. Neither the control nor the test subjects were on any treatment.

Eight ml of venous blood was collected aseptically from all the subjects by venipuncture after overnight fasting. Five ml of blood was allowed to clot, serum was separated immediately, blood sugar and lipid profile were estimated using Siemens dade dimension fully automated clinical chemistry analyzer. Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Prolactin (PRL) and Estradiol were measured by the Chemiluminescence Immunoassay (CLIA) in Centaur CP fully automated CLIA analyzer of M/s. Siemens Health Care Diagnostics India Ltd. Reagents, calibrators, controls and standards were procured from the same company.

The remaining three ml of blood was collected in sodium heparinized vacuutainers for detecting mutagen sensitivity analysis and set up the lymphocyte cultures using RPMI 1640 as the medium supplemented with 20% fetal bovine serum with 10µg/ml of Phytohemagglutinin and incubated. The cultures were treated with bleomycin (0.03 unit/ml) at S phase[10]. At 70th hour all the cultures were treated by colchicine to arrest the cell division at metaphase. For mutagen sensitivity, the slides were stained with Geimsa and look for frank chromatid breaks. The frequency of chromatid breaks was considered as a measure of an individual DNA repair capacity. For chromosome sensitivity analysis the mean break/cell (b/c) was calculated. The frequency of breaks was expressed as b/c for comparison. Any individual expression of a mean b/c value <0.8 was considered as hyposensitive, >0.8 was considered sensitive and >1.0 was considered hypersensitive. The data was computed and analyzed using SPSS 11.3 for Windows.

Results

The demographic and physiological findings of all the subjects are given in table 1, 2 and 3. The age of study subjects ranged from 15 to 35 years with a mean age of 25.87±5.23 years. The age of the control subjects ranged from 17 to 35 years with a mean age of 27.68±4.80. The birth order ranged from 1 to 9 and majority of the study subjects belonged to first birth order followed by third birth order. Majority of the study subjects (n=78; 67.24%) belonged to rural area followed by urban (n=24; 20.68%) and coastal area (n=14; 12.06%). One hundred and twelve test subjects had sedentary type of occupation and only 4 individuals have non sedentary type of occupation. Majority of the study subjects were married (n=79; 68.10%) and the rest (n=37; 31.89%) were unmarried. The duration of married life of these subjects ranged from 1 to 11 years with a mean duration of married life of 4.49 years. Parental Consanguinity was reported only in 8 out of 116 study subjects. Family history of infertility or sub-fertility is reported in 8 out of 116 study subjects. Five out of 116 study subjects reported family history of cancer among first or second degree relatives. Multiple X-ray exposures during their early period were reported in 7 subjects.

Table 1: Demographic and anthropometric characteristics of the study subjects and control subjects

	Category	Study subjects	Control subjects
		n (%)	n (%)
Age range	<21	21 (18.10)	6 (12.0)
	21-30	71 (61.20)	30 (60.0)
	>30	24 (20.69)	14 (28.0)
Birth order	1 to 3	84 (72.41)	40 (80.0)
	>3	32 (27.59)	10 (20.0)
Residence	Rural	78 (67.24)	29 (58.0)
	Urban	24 (20.69)	17 (34.0)
	Coastal	14 (20.06)	4 (8.0)
Nature of occupation	Sedentary	112 (96.55)	48 (96.0)
	Non sedentary	4 (3.44)	2 (4.0)
Parental consanguinity	Yes	8 (6.89)	1 (2.0)
	No	108 (93.10)	49 (98.0)
Marital status	Married	79 (68.10)	41 (82.0)
	Unmarried	37 (31.89)	9 (18.0)
Duration of married life	1 to 5	57 (49.14)	34 (82.93)
	>5	22 (18.97)	7 (17.07)
Economic status	High	12 (10.34)	27 (54.0)
	Medium	99 (85.34)	20 (40.0)
	Low	5 (4.31)	3 (6.0)
BMI	≤25	65 (56.03)	50 (100)
	>25	51 (43.96)	0 (0)

The clinical abnormalities observed in the study subjects with PCOS include, infertility (n=45; 38.79%), irregular menstruation (n=29; 25%), obesity (n=7; 6.03%), primary amenorrhea (n=5; 4.31%) and recurrent abortions (n=30; 25.86%). Majority of study subjects (n=63; 54.31%) attained menarche on or before 13 years of age, 48 subjects attained menarche on or after 14 years of age and the remaining 5 subjects did not attain menarche so far. Out of the 116 test subjects, irregular menstruation in 72, regular menstruation in 39 and no menstruation in five were reported. Nine test subjects had endometriosis and consumption of contraceptive drugs was reported in 12. The body mass index (BMI) of the study subjects showed 24.67±2.16 and the control subjects showed 22.50±1.76, this difference had statistical significance (t=6.289; *p*=<0.001). Fifty one out of 116 study subjects had a BMI above 25 (obese) and rest showed a BMI of less than 25.

The results of biochemical assessment of all the test subjects are given in table 4. The FBS of study subjects ranged from 84-148 with a mean of 123.57±14.82 mg/dL and the control subjects showed a mean FBS of 99.98±10.67. The difference was statically significant (t=10.171; *p*= <0.001). Normal serum total cholesterol was reported only in 44 study subjects and the remaining subjects were hypercholestrolimic (>200 mg/dL).

Table 2: Clinical abnormalities of the study subjects

Clinical abnormalities	Age range	n (%)
Infertility	<21	4 (3.45%)
	21-30	38 (32.76%)
	>30	3 (2.59%)
Irregular menstruation	<21	13 (11.21%)
	21-30	16 (13.79%)
	>30	0
Obesity	<21	2 (1.72%)
	21-30	5 (4.31%)
	>30	0
Primary amenorrhea	<21	2 (1.72%)
	21-30	3 (2.59%)
	>30	0
Recurrent abortions	<21	0
	21-30	9 (7.76%)
	>30	21 (18.1%)

Table 3: Physiological findings of the study subjects

	Category	n (%)
Family history of PCOS	Yes	6 (5.17)
	No	110 (94.83)
Family history of infertility/sub fertility	Yes	8 (6.89)
	No	108 (93.10)
Family history of cancer among first or second degree relatives	Yes	5 (4.31)
	No	111 (95.69)
Endometriosis	Yes	9 (7.76)
	No	107 (92.24)
Contraceptive drugs used	Yes	12 (10.34)
	No	104 (89.66)
H/o of X-ray exposure	Yes	7 (6.03)
	No	109 (93.96)
Menarche	≤13	63 (54.31)
	>13	48 (41.38)
	Not yet attained	5 (4.31)
Menstrual periods	Regular	39 (33.62)
	Irregular	72 (62.06)
	No	5 (4.31)

Table 4: Comparison of fasting blood sugar and lipid profile in test and control subjects

	Category	Mean	SD	t	*p*
Fasting blood sugar (mg/dL)	Test (n= 116)	123.57	14.82	10.171	<0.001
	Control (n=50)	99.98	10.67		
Total cholesterol(mg/dL)	Test (n= 116)	210.88	37.39	1.402	0.163
	Control (n=50)	202.68	26.84		
HDL cholesterol(mg/dL)	Test (n= 116)	36.37	7.32	-6.463	<0.001
	Control (n=50)	45.08	9.31		
LDL cholesterol(mg/dL)	Test (n= 116)	142.66	29.83	2.153	0.033
	Control (n=50)	131.36	33.61		
Triglyceride(mg/dL)	Test (n=116)	140.97	71.92	-0.087	0.931
	Control (n=50)	141.92	44.18		

Table 5: Comparison of reproductive hormones in test and control subjects

	Category	Mean	SD	t	*p*
FSH	Test (n=116)	25.83	6.60	15.745	<0.001
	Control (n=50)	9.64	4.65		
LH	Test (n=116)	51.78	13.71	18.409	<0.001
	Control (n=50)	14.90	5.32		
Prolactin	Test (n=116)	29.65	10.50	7.776	<0.001
	Control (n=50)	17.17	6.52		
Estradiol	Test (n=116)	69.01	33.61	0.965	0.033
	Control (n=50)	63.63	31.30		

Table 6: Comparison of mean b/c value of the test and control subjects

	Category	Mean	SD	t	*p*
Mean b/c value	Test (n=116)	0.8019	0.05	10.337	<0.001
	Control (n=50)	0.7166	0.05		

The HDL cholesterol values were equal or less than 35 mg/dL in 85 (73.27%) in the study subjects. LDL cholesterol values were higher and triglyceride values were lower in the majority of the study subjects. HDL and LDL cholesterol showed a significant difference between the study and the control subjects.

FSH, LH and prolactin showed a statistically significant difference with that of the control subjects. The study subjects showed a mean FSH value of 25.83±6.60 whereas in the control subjects the value was 9.64±4.65 (t=15.745; *p*=<0.001). The mean LH level of the test subject was 51.78±13.71 and in the control subject the mean value was14.90±5.32 (t=18.409; *p*=<0.001).The prolactin level was 29.65±10.50 and 17.17±6.52 (t=7.776; *p*=<0.001) respectively for the test and control subjects. The corresponding Estradiol level were 69.01±33.61 and 63.63±31.30 for the test and control subjects but the difference was not significant (t=0.965; *p*=0.336) (Table 5).

The mean break per cell (b/c) value of the study subjects was 0.8019 and that of the control subjects was 0.7166. This difference showed a statistical significance (*p*=<0.001) (Table 6). This indicates that the subjects with PCOS had a defective DNA repair capacity / DNA damage than the control subjects. More over the mean b/c value of the subjects were found to be increasing with increase in risk factors or with the severity of the risk factors.

Table 7: Distribution of Odds ratio

	Category				Total		*p*	OR	95% CI
	Test		Control						
	n	%	n	%	n	%			
Menarche >13	36	31.0	2	4.0	38	22.9	<0.001	10.80	2.49-46.89
LH (Abnormal)	102	87.9	8	16.0	110	66.3	<0.001	38.250	14.941-97.924
Prolactin (Abnormal)	76	65.5	6	12.0	82	49.4	<0.001	13.93	5.47-35.48
FBS (Abnormal)	92	79.3	9	18.0	101	60.8	<0.001	17.46	7.46-40.85
LDL (Abnormal)	85	73.3	23	46.0	108	65.1	0.001	3.219	1.61-6.42
HDL (Abnormal)	35	30.2	4	8.0	39	23.5	0.002	4.97	1.66-14.87
b/c (Abnormal)	78	67.2	4	8.0	82	49.4	<0.001	23.605	7.914-70.405

Table 8: Logistic regression analysis

	B	S.E.	Wald	df	*p*	Exp(B)
Menarche >13	3.835	1.464	6.862	1	0.009	46.305
Prolactin (Abnormal)	2.593	0.784	10.948	1	0.001	13.370
FBS(Abnormal)	2.538	0.728	12.144	1	0.000	12.658
LDL (Abnormal)	0.999	0.716	1.945	1	0.163	2.716
b/c value	1.702	0.823	4.278	1	0.039	5.482
LH (Abnormal)	2.508	0.726	11.942	1	0.001	12.280
HDL (Abnormal)	1.359	0.891	2.325	1	0.127	3.891
Constant	-11.087	2.428	20.855	1	0.000	0.000

Odds ratio for the following variables indicate that subjects who attained menarche >13 years, increased LH, Prolactin, FBS and LDL-C, decreased HDL-C and increased b/c value had significant contribution for PCOS (Table 7). Logistic regression analysis revealed that the above parameters were also significant risk factors for developing PCOS (Table 8).

Discussion

In young women with PCOS, multiple risk factors for CVD, including metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), dyslipidemia, abdominal obesity, and hypertension[11]. Dyslipidemia is very common in PCOS patients[12] and may present with different patterns, including low levels of high-density lipoprotein cholesterol (HDL-C), increased values of triglycerides and total and low-density lipoprotein cholesterol (LDL-C), as well as altered LDL quality. These different patterns may be related to the associated effects of insulin resistance and hyperandrogenism that combine with environmental (diet, physical exercise) and genetic factors[13,14].

Differences between PCOS and control women exist in several CVD risk factors, which often are more profound in obese PCOS women. The available evidence shows that different lipid patterns may be present in women with PCOS. In addition, differences between diverse ethnic and geographical backgrounds cannot be fully explained by variations in body weight alone[13,14] but are likely to depend on the combination of genetic, environmental, and hormonal factors. In support of this, non-obese women with PCOS also can have elevated levels of lipoprotein (a)[15]. In the present study, subjects with PCOS showed a statistically significant level of increased biochemical and hormonal risk factors and also showed a defective DNA repair capacity/DNA damage.

The present study observed a defective DNA repair proficiency among subjects with PCOS than the control; this supports the hypothesis that the DNA damage may expand the prognostic power of established biomarkers for the detection and the progression from metabolic syndrome to T2D mellitus and CVD[7,16]. Moreover, a better understanding of the association between DNA repair mechanism

and cardiometabolic disease need to carefully consider a series of clinical and confounding factors such as the quality of the metabolic control, the type of diabetes, the duration of the disease, the influence of genetic polymorphisms, the drugs and radiation imaging treatment used for therapy. To gain more insights in the role of DNA repair capacity in cardiometabolic disease, need large population-based studies.

However, more research is needed to provide a greater understanding of the interaction between hyperandrogenism, insulin resistance, abdominal adiposity in PCOS and the DNA repair capacity. Overall, there remain considerable gaps in the literature which require further research. Insights in these areas across the phenotypes will also assist in understanding the underlying pathophysiology of metabolic features of this condition. Future research in PCOS should identify optimal risk prediction tools forT2DM and CVD and optimal definition and utility of the metabolic syndrome for disease prediction. This will help clarify the optimal timing and determine best screening practices for minimizing cardiometabolic morbidity and mortality. The present study demonstrated that women with PCOS have an elevated prevalence of hormonal imbalances; obesity, impaired glucose tolerance (IGT) and metabolic syndrome. Ultimately, classification of the metabolic complications for each phenotype will provide an evidence base for screening of metabolic risk upon diagnosis of PCOS and may guide optimal treatment to prevent metabolic complications of PCOS.

Conclusions

In the present study dyslipidemia, abnormal hormonal level and increased genetic instability were observed in PCOS. Alarmingly, IGT and metabolic syndrome are highly prevalent among PCOS subjects with age and weight gain worsening glycemic control. Lifestyle management is recommended for primary CVD prevention, targeting low-density and non-high-density lipoprotein cholesterol and adding insulin-sensitizing and other drugs if dyslipidemia or other risk factors persist. Women with polycystic ovary syndrome should modify their lifestyle by changing their diet and exercise routine to reduce weight and improve ovarian function. This point is of particular interest in relation to evidence showing that a range of healthy lifestyle factors, including exercise, are associated significantly with reduced DNA damage.

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