Risk Factors for Cardiovascular Disease in Infertile women with Polycystic Ovarian Syndrome

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Abstract

A study was conducted to evaluate the risk of cardiovascular disease (CVD) in infertile women with polycystic ovary disease. There were 46 test subjects in the age group of 20 to 40 years and 40 age matched healthy controls. Serum lipid profile, fasting blood sugar (FBS) and reproductive hormones namely Leutinizing hormone (LH), follicle stimulating hormone (FSH), Prolactin and Estradiol were estimated in this era of all the subjects after obtaining their informed consent. Cytoklinesis-block micronuclei (CBMN) was also evaluated and attempts were made to correlate the findings with the risk of CVD. Decreased HDL and elevated levels of all other biochemical and endocrinological parameters were observed in the test subjects. A significantly elevated level of micronuclei (MN) in the test subject along with the above parameters and body mass index (BMI) is suggestive of increased risk of CVD in PCOS.

Key wards

Polycystic ovary disease, cardiovascular disease, fasting blood sugar, body mass index, lipid profile, infertility and reproductive hormone, Somatic DNA damage.

Introduction

Polycystic ovarian syndrome (PCOS) is a major cause of infertility in women affecting 6−10% of women of childbearing age [1], [2]. It is related to the absence of ovulation (anovulatory infertility). The metabolic profile noted in women with PCOS is similar to the insulin resistance syndrome, a clustering within an individual of hyperinsulinemia, mild glucose intolerance, dyslipidemia, and hypertension. Insulin resistance is thought to play a role in the pathogenesis of PCOS, and is often exacerbated by co-existent obesity. Dyslipidaemia may be the most common metabolic abnormality in PCOS, with a prevalence of up to 70% by the National Cholesterol Education Program criteria [3].

Clinical science and symptoms in women with PCOS are similar to those with metabolic cardiovascular syndrome [3]. Although there is a paucity of data providing evidence for cardiovascular events in women affected by PCOS during their fertile years [4], an increased prevalence of cardiovascular risk factors has been well documented [5].

PCOS is classically associated with an atherogenic lipoprotein profile, characterized by elevated triglyceride-rich lipoproteins, accumulation of small dense low density lipoprotein (LDL) and depressed high density lipoprotein (HDL). All these changes were reported to be due to insulin resistance, although elevated androgens may contribute to small HDL size by stimulating hepatic lipase activity [6]. Early subclinical atherosclerotic disease, as evidenced by carotid intimal media thickness [7] and increased coronary artery calcification [8], [9] were reported in women with PCOS. Several studies had demonstrated abnormalities in nontraditional markers of CVD related to insulin resistance and obesity in PCOS, such as those involving homocysteine and markers of oxidative stress [10], C-reactive protein [11] and adiponectin [12], plasminogen activator-1, Von Willebrand factor 1, endothelin-1 [13] had shown their association with PCOS. Studies had shown the association of PCOS with hyperinsulinemia and insulin resistance which may lead to CVD [14], [4].

Recently, obesity was reported to be associated with lower arterial distensibility, a measure of the elastic properties of the vessel wall, and an index of circulatory function relevant to the

atherosclerotic process. Normal weight women with PCOS would have the same degree of cardiovascular risk as overweight women with PCOS and that the persistence of PCOS symptomatology over time would be associated with increased cardiovascular risk [15]. Obesity imparts insulin resistance and exacerbates reproductive and metabolic features of PCOS. Two-fold increase in the risk of CVD and a five-fold increase in the risk of type 2 diabetes were reported in infertile women with PCOS, particularly those with age ≥25 years or with central obesity (a waist hip ratio of ≥0.85) were at higher risk of developing metabolic syndrome [16].

Studies on the effect of DNA damage and defective repair mechanism, metabolic and biochemical alterations which predisposes infertile women with PCOS to CVD are scanty from this area and hence the present study was undertaken to evaluate the genetic, metabolic, hormonal and biochemical alternations which predisposes infertile women with PCOS to CVD.

Materials and Methods

Forty six infertile women in the age group of 20-40 years with a clinical diagnosis of PCOS referred from various gynaecology and infertility centres of Kerala formed the test group of the study. Forty asymptomatic, age matched healthy fertile women formed the control group. Informed consents were obtained from all the subjects of the study according to the norms laid down by the Institutional Ethical Committee.

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 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 Chemi Luminescent Immuno Assay (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 quantifying the extent of somatic DNA damages by Cytokinesis-block micronuclei (CBMN) assay. Two ml of lymphoprep (pharmacia) added to a 10 ml centrifuged tube and overlaid 3 ml of blood sample to the tube and centrifuged at 1,000 rpm for 10 min. Drawn off the lymphocyte

layer and transferred to a 10 ml tube. Suspended the cell pellet in RPMI 1640 medium and centrifuged for 10 minutes and removed the supernatant and repeated the above step. Peripheral lymphocyte culture was performed as described by Moorhead et al. [17]. The CBMN test was done using the cytochalasin B technique described by Fenech [18]. The lymphocytes were cultured in sterile bottles using RPMI 1640 medium containing 15% fetal calf serum. Lymphocyte cultures were prepared for each subject. 5 ml RPMI 1640 supplemented with 100 units/ ml penicillin, 100 lg/ml streptomycin, 10% fetal bovine serum and 1% phytohemagglutinin. At 44 h after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, final concentration, 4.5µg/ml). The total incubation time for all cultures was 72 h. After incubation, the cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored at 100X magnification. The numbers of micronuclei per 1,000 binucleated cells were recorded. The data was computed and analyzed using SPSS 11.3 for Windows.

Results

The demographic and anthropometric observations were recorded in Table 1. The age of study subjects ranged from 22 to 40 years with a mean age of 28.54±4.77 years. The age of the control subjects ranged from 23 to 35 years with a mean age of 29.80±2.92, this difference had no statistical significance ($t = -1.446$; $p= 0.152$). The birth order of study subjects ranged from 1 to 10 and the difference had no statistical significance between the test and control subjects. Majority of the study subjects (n=32; 69.6%) belonged to rural area followed by urban area (n= 14; 30.4%). Majority of the study subjects (n= 32; 69.6%) were Hindus followed by Christians (n=7; 15.2%) and remaining were Muslims (n= 7; 15.2%). Twenty four of the study subjects (52.2%) were graduates or above in education and the remaining were up to higher secondary (47.8%). 78.3% of the study subjects were unemployed where as 21.7% were employed.

Thirty five study subjects (76.1%) had regular menstrual periods and 11(23.9%) had irregular menstrual periods. 84.8% (n=39) of subjects had menarche at the age of ≤13 and the rest (n= 7) had at the age of ≥14. Among 46 study subjects 23 (50%) were obese and among 40 control subjects 4 (1%) had over weight. The difference had statistical significance ($p<0.001$).

The BMI of the study subjects showed 24.77±2.93 and the control subjects showed 22.93±1.96, this difference had statistical significance (t =3364; p= 0.001). The Body mass index (BMI) of the infertile women with PCOS was higher than that of the age matched control subjects.

Only one among the study subjects had the family history of cancer among the first and second degree relatives and history of chronic illness. Remaining (n= 45; 97.8%) had no such histories and illness. Two of the study subjects (4%) had family history of infertility or sub fertility. Majority of the study subjects and all the control subjects had no previous history of X-ray exposure. Consanguinity and duration of married life of the study subjects and control subjects were tested. Thirty one study subjects (67.4%) had duration of married life ≤5 years. Majority of the study subjects (n= 40; 87.0%) and control subjects (n= 32; 80%) were not used any contraceptive drugs. Majority of the study subjects were belonged to low/medium economic status (n= 34; 73.9%) and the rest were belonged to high economic status (n= 12; 26.1%). The difference had statistical significance among the study subjects and control subjects. Majority of the study subjects (n= 42; 91.3%) and control subjects (n= 38; 95%) had no history of parental consanguinity.

Comparison of the mean values of the biochemical and hormonal parameters of the test and control subjects are given in Table-2. The following biochemical evaluations revealed a statistically significant difference between the study subjects and the control subjects. The FBS of study subjects ranged from 84-147 with a mean of 123.33±17.05 mg/dL and the control subjects showed a mean FBS of 100.48±11.50; (t= 7.174; p<0.001). Normal serum total cholesterol was reported only in 15 study subjects and the remaining subjects were hypercholesterolimic. The total cholesterol of study subjects ranged from 114-262 with a mean of 210.26±32.27mg/dl and the control subjects had a mean value of 180.10±17.03; (t= 5.301; p= 0.001).

The HDL cholesterol values were equal or less than 35 mg/dL in 18 study subjects. The HDL cholesterol of study subjects ranged from 21-51mg/dL with a mean of 36.59±6.80 mg/dL and that of control subjects showed a mean of 48.50±7.23 mg/dL; (t= -7.867; p= 0.001). The LDL cholesterol values of study subjects ranged from 75-199 mg/dL with a mean value of 143.78±29.22 and that of control subjects showed a range of 63-151 mg/dL with a mean value of 117.53±21.22; (t= 4.705; p= 0.001). The total triglyceride of study subjects ranged from 71-

669 with a mean of 157.04±98.25 mg/dl and the control subjects had a mean value of 116.35±20.21; (t= 2.571; p= 0.012).

The following hormones also revealed a statistically significant difference between the study subjects and the control subjects. FSH, LH, prolactin and estradiol showed a statistically significant difference. The study subjects showed a mean FSH value of 26.29±6.44 whereas in the control subjects the value was 9.39±5.75 (t = 12.743; p= 0.001). The mean LH level of the test subjects was 53.94±13.19 and in the control subjects the mean value was 14.78±5.67 (t= 17.421; p= 0.001). The Prolactin level was 27.93±9.02 and 16.77±6.74 (t= 6.423; p= 0.001) respectively for the test and control subjects. The Estradiol level was 70.00±29.87 for the test and 28.04±9.88 for the control subjects and the difference was statistically significant (t= 8.484; p= 0.001).

Fasting blood sugar (FBS), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C) and triglyceridesubjects (TG) were significantly elevated in the test group compared to the control group. High density lipoprotein cholesterol (HDL-C) of the test group was significantly lower than that of the control. The reproductive and infertile hormones like follicle stimulating hormone (FSH), Luetinizing hormone (LH), Prolactin and Estradiol of the test subjects was significantly higher than the controls.

The mean CBMN frequency of the study subjects (n=46) was 14.50 ±1.60 and that of the control subjects (n=40) was 10.01±1.25. This difference showed statistical significance (t=14.353; p=0.001) (Table 3). Moreover a positive correlation between the number of risk factors and the extent of DNA damages was also observed. Linear regression analysis (Table 4 & 5) revealed that the variation CBMN frequency is determined by the independent variables like BMI, FBS, Total Cholesterol, LDL, TG, HDL, Prolactin, Estradiol, FSH and LH. 64.3% (R= 0.802, R – Square = 0.643) of the variation in CBMN frequency is determined by these variables.

Table 1. The demographic and anthropometric characteristics

Category		Study		Control	
		Number	Percentage	Number	Percentage
Age Range	20-24	5	10.9	1	2.5
	25-29	30	65.2	17	42.5
	30-34	3	6.5	18	45.0
	35-40	8	17.4	4	10.0
Birth Order	1	8	17.4	15	37.5
	2	10	21.7	10	25.0
	3	10	21.7	7	17.5
	≥4	18	39.2	8	20.0
Place of residence	Rural	32	69.6	27	67.5
	Urban	14	30.4	13	32.5
Religion	Hindu	32	69.6	28	70.0
	Christian	7	15.2	6	15.0
	Muslim	7	15.2	6	15.0
Educational achievements	Up to higher secondary	22	47.8	24	60.0
	Degree and above	24	52.2	16	40.0
Occupation	Unemployed	36	78.3	26	65.0
	Employed	10	21.7	14	35.0
Menstrual periods	Irregular	11	23.9	2	5.0
	Regular	35	76.1	38	95.0
Menarche	≤13	39	84.8	36	90
	≥14	7	15.2	4	10

Table 2. Comparison of the biochemical and hormonal profile of the test and control subject

Category		N	Mean	Sd	t	p
Age	Study	46	28.54	4.77	-1.446	.152
	Control	40	29.80	2.92		
BMI	Study	46	24.77	2.93	3364	.001
	Control	40	22.93	1.96		
FBS	Study	46	123.33	17.05	7.174	.001
	Control	40	100.48	11.50		
Total cholesterol	Study	46	210.26	32.27	5.301	.001
	Control	40	180.10	17.03		
HDL	Study	46	36.59	6.80	-7.867	.001
	Control	40	48.50	7.23		
LDL	Study	46	143.78	29.22	4.705	.001
	Control	40	117.53	21.22		
TG	Study	46	157.04	98.25	2.571	.012
	Control	40	116.35	20.21		
FSH	Study	46	26.29	6.44	12.743	.001
	Control	40	9.39	5.75		
LH	Study	46	53.94	13.19	17.421	.001
	Control	40	14.78	5.67		
Prolactin	Study	46	27.93	9.02	6.423	.001
	Control	40	16.77	6.74		
Estradiol	Study	46	70.00	29.87	8.484	.001
	Control	40	28.04	9.88		

Table.3 CBMN frequency of the test and control subjects

	Category	N	Mean	Sd	T	p
CBMN frequency	Study	46	14.50	1.60	14.353	.001
	Control	40	10.01	1.25		

Table.4 Linear regression analysis

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
FSH	1.190	3.810	.098	1	.755	3.287
LH	4.573	1.575	8.428	1	.004	96.850
prolactin	2.490	1.473	2.857	1	.091	12.062
Estradiol	1.204	1.512	.634	1	.426	3.334
Economicstatus	-1.235	1.639	.568	1	.451	.291
Obesity	.081	2.412	.001	1	.973	1.084
cbmn	1.702	.704	5.840	1	.016	5.483
Constant	-4.811	5.081	.897	1	.344	.008

a. Variable(s) entered on step 1: FSH, LH, Prolactin, Estradiol, Economic status, Obesity, CBMN.

Table.5 Linear regression analysis

Coefficients[a]

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	6.015	2.873		2.093	.040
FBS	.014	.013	.101	1.142	.257
Total cholesterol	-.001	.011	-.015	-.118	.907
HDL	-.019	.033	-.065	-.566	.573
LDL	.001	.013	.007	.052	.959
TG	.004	.003	.122	1.412	.162
FSH	.049	.035	.190	1.389	.169
LH	.053	.018	.443	3.032	.003
Prolactin	.000	.023	-.002	-.023	.981
Estradiol	.003	.008	.032	.331	.741
BMI	.092	.079	.092	1.167	.247

a. Dependent Variable: CBMN frequency

Discussion

Paradisi et al [19] examined the prevalence of risk markers of subclinical CVD in women with PCOS. There was evidence for impaired endothelial function, an early marker of atherosclerosis, in young women with PCOS. An increased risk of CVD in women with presumed features of PCOS was also reported [20]. Carotid intima-media thickness, another marker associated with increased prevalence of stroke and myocardial infarction was also increased in premenopausal women with PCOS [21]. Leslee et al [22] suggest that postmenopausal women with clinical features of PCOS have an elevated risk of CVD or

nonfatal MI that is independent of their underlying clinical risk, suggesting that PCOS-related protracted hyperandrogenism may be one mechanism responsible for their adverse cardiac risk.

A study by Azziz et al. [23] demonstrated that overweight or obesity affects approximately 60–80% of PCOS patients. In obese women, excess insulin and androgens contributed to the development of the PCOS and metabolic syndrome [24]. In the present study 50% (n= 23) of the subjects in the test group were obese. In a study the proportion of PCOS patients with a BMI above 23 kg/$m^2$was 34.63% [25] where as in the present study 50% (n= 23) of test subjects had higher BMI. This is in agreement with the study by Kavita et al [16] who reported an increased prevalence of metabolic syndrome in women with PCOS presenting with infertility.

The main pathophysiological components of PCOS are gonadotropic dysfunction and insulin resistance. It has been found that both of these components are related to the BMI [26]. Kavita et al. [16] reported a low prevalence of elevated FBS in patients with PCOS. A high glucose level can indicate insulin resistance, a diabetes-related condition that contributes to PCOS. The present study showed an overall prevalence of elevated levels of FBS in women with PCOS.

A study by suhail et al. [27] 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 in 67.4% (n= 31), LDL in 47.8% (n= 22) and TG in 54.3% (n= 25) and decreased levels of HDL in 93.5% (n=43) was observed.This is suggestive of an increased risk for CVD in patients with PCOS.

Lavanya et al. [28] reported that only 17.18% of PCOD patients had elevated LH levels. According to the present study 91.3% (n= 42) of PCOD patients showed elevated LH levels. Irfan et al. [29] reported that in 15-20% of the PCOS patients mild elevated prolactin levels can be functionally found without prolactinoma. In the present study 63% (n=29) of study subjects had elevated level of prolactin. Johanna et al [30] demonstrated that FSH levels are lower in infertile women with PCOS women. The present study is not in agreement with this report as the mean FSH level in the test group was significantly higher than that of the controls.

Yesilada et al. [31] reported that women with PCOS had a high incidence of genomic instability, and this condition was positively correlated with the hirsutism score, BMI, LH, total testosterone and insulin levels and was negatively correlated with Sex hormone binding globulin (SHBG). An elevated level of genomic instability (greater number MN and chromosome mal-segregation) present in women with PCOS was positively correlated with the BMI and insulin resistance levels [32]. An association between increased micronuclei (MN) frequency and polycystic ovary syndrome was also demonstrated [33]. The simplicity, rapidity and sensitivity of the CBMN assay may provide a useful tool for screening of the infertile women with PCOS. In order to explore this potential, further studies are needed to investigate in more depth about the role of MN in predicting the risk of CVD in these patients.

The study clearly indicates that there is an increase in DNA damage in infertile subjects with PCOS. The higher the number of risk factors, higher will be the chance of developing CAD in infertile women with PCOS. Lifestyle modification with diet and exercise, lowering serum lipids and blood sugar will reduce the risk of metabolic syndrome and CAD in infertile women with PCOS.

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