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RESEARCH ARTICLE

Predisposing factors in Polycystic Ovarian Syndrome for Cardiovascular disease

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Abstract

Polycystic ovarian syndrome (PCOS) is a heterogeneous, multifactorial, complex genetic and endocrine disorder, characterized by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries. It is a common endocrinopathy affecting 6–10% of reproductive aged women. 28 infertile women were selected as study subjects and 25 asymptomatic healthy fertile women as control subjects in this study. The goal of the present study was to evaluate the predisposing risk factors leading to cardiovascular disease (CVD) in subjects with polycystic ovarian syndrome. Various biochemical parameters such as blood sugar, lipid profiles and hormones such as Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were assessed in the sera of all these subjects. Decreased high density lipoprotein (HDL) and elevated levels of all other biochemical and endocrinological parameters were observed in the study subjects. Elevated level of break per cell value (b/c) in the study subjects along with the above parameters and raised body mass index (BMI) is suggestive of increased risk of CVD in PCOS. The findings imply that there is a strong evidence for increased risk of developing CVD in subjects suffering from PCOS. Lifestyle modifications by increasing physical exercise and dietary control will help in modifying the CVD risk factors in PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous, multi-factorial, complex genetic and endocrine disorder, characterized by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries (Witchel, 2006). It is a common endocrinopathy affecting 6–10% of reproductive aged women (Azziz et al., 2004).

PCOS is associated with 10 fold risk for developing type 2 diabetes in adulthood (Nestler, 2008) and 2 fold increased rate of metabolic syndrome (Essah et al., 2007). According to a recent study, more than half of adolescent PCOS patients had high density lipoprotein levels below the normal reference range for their sex (Bekx et al., 2010) which is a strong predictor of future cardiac risk. PCOS is the most common endocrinopathy in women of reproductive age, with a prevalence ranging from 5 to 10% in the general population and almost 30% among obese women (Azziz et al., 2004; Alvarez et al., 2006).

PCOS is associated with an approximately 7-fold increased risk of type 2 diabetes mellitus (DM) (Jakubowski, 2005). Insulin resistance (IR) and pancreatic β cell dysfunction are major risk factors for the development of type 2 DM. Defects in insulin action and insulin secretion are critical determinants in the pathogenesis of glucose intolerance in PCOS and both are influenced by genetic and environmental factors (Carmina, 2009). Women with PCOS are predisposed to develop Impaired Glucose Tolerance (IGT) and type 2 diabetes mellitus (Legro et al., 1999). Obesity, insulin resistance, and impaired pancreatic cell function contribute to this predisposition. In addition to these recognized factors, racial origin and the presence of type 2 diabetes in a first degree relative influence the risk of glucose intolerance, although this issue has been examined in a limited number of studies involving women with PCOS (Yildiz et al., 2003).

Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. These changes may be manifested by the elevation of serum total cholesterol (TC), low density lipoprotein (LDL) cholesterol and triglycerides (TG) concentration and a decrease in the high density lipoprotein (HDL) cholesterol concentration. As compared to women without PCOS, 85% of PCOS women have dyslipidemia characteristic of the metabolic syndrome. Obesity has an important influence on the lipid profile with approximately 50% of patients with PCOS being overweight or obese with abdominal fat accumulation. Insulin is positively correlated with total cholesterol, LDL and TG, and negatively correlated with HDL in IR patients. Dyslipidemia was found to be a major prognostic risk factor for cardiovascular disease (Legro et al., 1999).

PCOS is classically associated with an atherogenic lipoprotein profile, characterized by elevated triglyceride rich lipoproteins, accumulation of small dense LDL and depressed 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 (Rajkhowa et al., 1997). Early subclinical atherosclerotic disease, as evidenced by carotid intimal media thickness (Talbott et al., 2000) and increased coronary artery calcification (Talbott et al., 2004) were reported in women with PCOS. Studies had shown the association of PCOS with hyperinsulinemia and IR which may lead to CVD (Carmina, 2009; Shaw et al., 2008).

Menstrual cycle irregularity may be a marker of metabolic abnormalities predisposing women to an increased risk for cardiovascular disease (CVD). The most well known correlation between metabolic syndrome and reproductive disorders is in women with PCOS. IR is the most common metabolic abnormality in PCOS patients followed by obesity and dyslipidemia with an incidence of 31.5% for the metabolic syndrome (Zeyneloglu et al., 2006). The major risk factors leading to the metabolic syndrome or cardiovascular dysmetabolic syndrome are physical inactivity and an atherogenic diet, and the cornerstone clinical feature is abdominal obesity or adiposity (Lahbadia et al., 2008).

The PCOS is a familial disorder, but the genetic basis of the syndrome remains controversial. Determining the mode of inheritance of this syndrome is difficult because there has been no clearly described male phenotype and because it is a disorder that affects principally women of reproductive age there is now a larger focus on the management of the metabolic outcomes of PCOS, essentially through lifestyle intervention to achieve weight loss and developing physical activity (Carey et al., 1993). There are only few studies concerning the association between phenotypic expression, body make up and PCOS, and association with the processes of sexual maturation, growth and various environmental factors (stress, nutrition, physical activity and other factors). There is a lack of information about further PCOS development and prognosis, considering the environmental and individual factors. 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 endocrinological, biochemical and genetic influence (Zabuliene and Tutkuviene, 2010).

Materials and Methods

Twenty eight infertile women in the age group of 17 to 37 years with a clinical diagnosis of PCOS referred from various gynecology and infertility centers of Kerala formed the test group of this study. Twenty five asymptomatic age matched healthy fertile women in the age group of 17 to 35 years 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 venepuncture after overnight fasting. 3 ml of blood was collected in sodium heparinised vacuutainers and mutagen induced bleomycin sensitivity assay was performed as described by Hsu et al., 1997. The remaining 5 ml of blood was allowed to clot and serum separated immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer. The

level of the serum lipid peroxide marker, malondialdehyde (MDA) was determined using thiobarbituric acid as main reagent by measuring these values on photoelectric colorimeter at 540nm.

For mutagen sensitivity analysis, set up the lymphocyte cultures using RPMI 1640 as the medium supplemented with 15 % fetal bovine serum, 10µg/ml phytohaemagglutinin. 0.03 units/ml of bleomycin treatment was given 6 hrs before harvesting to induce chromosome breakage. At the end of 70^{th} hour, the culture was treated by colchicine (0.04µg/ml) to arrest the cell division at metaphase. Then the culture was incubated for 72 hours at 37^0C. For mutagen sensitivity, the slides were stained with Giemsa and look for chromosomal lesions such as breaks, gaps, accentric fragments, ring chromosome etc, were also scored. The frequency of chromatid breaks were considered as a measure of an individual's DNA repair capacity. For chromosome sensitivity analysis the mean number of break per cell (b/c) was calculated. The frequencies of breaks were expressed as b/c for comparison. Any individual expression <0.8 was considered hyposensitive, between 0.8 and 1.0 as sensitive and those >1.0 was considered hypersensitive. A minimum of 100 metaphases per culture was scored and data were analyzed.

Result

Table (1)
Demographic and anthropometric characteristics of the Study and Control subjects

Category		Study		Control	
		N	%	N	%
Age range	≤20	7	25%	4	16%
	21 to 30	8	28.57%	15	60%
	>30	13	46.42%	6	24%
Birth order	1 to 3	27	96.42%	19	76%
	>3	1	3.57%	6	24%
Place of residence	Rural	13	46.42%	15	60%
	Coastal	2	7.14%	2	8%
	Urban	13	46.42%	8	32%

The demographic and anthropometric characteristics of the study and the control subjects were given in the table 1. The age of study subjects in the current study ranged from 17 to 37 years with a mean age of 28. The age of the control subjects ranged from 17 to 35 years with mean age of 27.08. The birth order of study subjects ranged from 1 to 5 and the majority of study subjects belonged to group 1 to 3 followed by others. Majority of the study subjects belonged to both rural (n=13; 46.42%) and urban (n=13; 46.42%) followed by coastal area. 16 subjects had sedentary type of occupation and 12 have non- sedentary type of occupation. The mean b/c value (0.802) was higher in urban area. Among the twenty eight study subjects, 16 subjects (57.14%) had irregular menstrual period and 12 subjects (42.85%) had regular menstrual period. The irregular menstrual period had higher chance of infertility. Also, 13 of the study subjects showed (46.42%) menarche at the age of ≤12 and 15 subjects showed (53.57%) menarche at the age of ≥13. The body mass index of the infertile women with PCOS was found to be higher than that of the age matched control subjects which indicate that BMI is commonly associated with PCOS.

The following biochemical evaluations revealed a statistically significant difference between the study subjects and the control subjects. The mean total cholesterol was found to be 195.82 mg/dl in study subjects and 190.28 mg/dl in the control subjects and this difference had statistical significance. The HDL value was found to decrease with increasing b/c value in the PCOS patients. A statistically significant difference was observed among the study and control subjects. In the case of LDL value and triglyceride, it was found to be increased with increasing b/c value.

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 mean value of FSH among the study subjects and control subjects was 24.89 IU/mL and 9.72 IU/mL respectively. The follicle stimulating hormone was increased in PCOS patients. The prolactin level was 27.35 ng/ml and 17.11 ng/ml respectively for the study and control subjects. The mean LH level was 52.61 IU in study subjects and 15.9 IU in control subjects. The LH was also found to be increased in PCOS patients.

Table (2)
Distribution of mean b/c value and MDA value among the Study and Control Subjects

Category	Number	Mean b/c value	MDA value
Study	28	0.7919	1.6
Control	25	0.7203	0.86

Among the 28 study subjects the mean break per cell value was found to be 0.7919 and that of control subjects was 0.7203. This indicates that the subjects with PCOS had a defective DNA repair capacity / DNA damage than the control subjects. Thus there is an increased chance of DNA damage and defective DNA repair system with increased severity of risk factors among the PCOS subjects than control subjects.

Discussion

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy affecting 6–10% of reproductive aged women (Azziz et al., 2004) and manifested by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries in its complete phenotype (Azziz et al., 2006). 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 (Carmina, 2009). More than 50% of PCOS patients have metabolic syndrome, including obesity, insulin resistance and dyslipidemia.

With increased adiposity in two thirds of American PCOS women (Azziz et al., 2009), the degree to which obesity and PCOS interact to promote premature atherosclerosis and increase cardiovascular mortality is a worldwide concern (Shaw et al., 2008; Christian et al., 2003).

PCOS is characterized by chronic anovulation (failure or absence of ovulation) and hyperandrogenism (excessive production of male hormones in women) with clinical manifestations of irregular menstrual cycles, infertility, hirsutism, and acne (Kahn and Gordon, 1999) which is a common condition affecting women of reproductive age in 5 to 10% (Carmina and Lobo, 1999). Schuring et al., (2008) reported that the elevated GnRH pulses further increase LH level and reduce FSH, which converts excess androgen into estrogens via aromatase activity in normal women. The present study is not in agreement with this report as the mean FSH level in the study group was significantly higher than that of the controls.

Charnvises et al., (2005) reported that menstrual irregularity is the most common gynecological presentation of PCOS, oligomenorrhea being observed in approximately 85–90% of women with PCOS, while as many as 30–40% of amenorrheic patients have PCOS. This fact is supported by the present study that 57.14% of study subjects showed irregular menstrual periods. Jamal and Ozgur, (2006) reported that long term hyperinsulinemia in humans, as is the case in PCOS patients, stimulates leptin secretion from adipose tissue (Conn et al., 2000). Although both insulin resistance and hyperinsulinemia have significant pathogenic roles in PCOS, women with hyperinsulinemia are not necessarily all hyperandrogenic and only 52% of those with type 2 diabetes mellitus have clinical manifestations of androgen excess.

Essah et al., (2008) reported that there is some evidence of a more atherogenic lipid profile (increased levels of TC, LDL cholesterol and triglycerides, and decreased levels of HDL cholesterol) among women with certain ovulatory disorders, specifically among women with PCOS. The present study also observed elevated levels of triglyceride and low density lipoprotein and decreased level of high density lipoprotein. These findings imply the fact that women with PCOS have certain predisposing factors for developing cardiovascular disease.
Balen et al., (1995) and Kiddy et al., (1990) reported that obesity and excess weight are major chronic diseases in Western world countries. Obesity increases hyperandrogenism, hirsutism, and infertility and pregnancy complications both independently and by exacerbating PCOS. Thus the present study suggests that obesity has become one of the major factors not only for both metabolic syndrome and cardiovascular disease; but also for PCOS.

Conclusion

The clinical abnormalities observed in the study subjects with PCOS include irregular menstruation, obesity and type 2 diabetets mellitus. The mean break per cell value of study subjects were significantly higher than that of control subjects, indicating that the subjects with PCOS showed a defective DNA repair capacity. The anthropometrics and demographic characteristics of the present study revealed that the life style factors like lack of physical exercise, increased lipids, obesity and poor glycemic control may contribute significantly to PCOS. The present study found a diabetogenic pattern in PCOS subjects to develop IGT or Type 2 DM by the later stages of life. The findings imply that there is a strong evidence for increased risk of developing CVD in subjects suffering from PCOS. Life style modification by increasing physical exercise and dietary control will help in modifying the CVD risk factors in PCOS.

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