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Study of levels of malondialdehyde, super oxide dismutase and hsCrp in serum of non-obese patients with polycystic ovarian syndrome

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a heterogenous disorder with diverse clinical features. PCOS patients are known to develop long term complications like cardiovascular disease (CVD), type 2 diabetes mellitus, endometrial cancer, breast cancer but increasing evidence shows presence of CVD in many PCOS patients. The key role in the pathophysiology of this disease is the presence of oxidative stress which is an important component of the cardiometabolic risk and is seen in obese women with PCOS. Presence of inflammation is indicative of future development of CVD. PCOS is a condition of low grade inflammation. This study was taken up to study the oxidant status and hsCrp levels in non-obese PCOS women.

Aim: To measure the levels of MDA, SOD and hscrp in serum of non-obese PCOS patient's ànd non obese women without PCOS.

Materials and Method: A cross sectional study was conducted from December 2011 to December 2012. 60 age and BMI matched subjects were included in the study between 25 -35 years of age group; 30 non obese pcos women as cases and 30 non obese normal women as controls. In all subjects the serum levels of MDA, SOD, hscrp was measured by Nadiger et al method, Marklund and Marklund method and immunoturbidometry method respectively.

Results: Decreased serum levels of SOD and increased serum levels of MDA and hscrp was seen in PCOS women compared to normal women.

Conclusion: Development of CVD in non-obese PCOS women is due to presence of oxidative stress and inflammation as risk factors.

Keywords: Cardiovascular Disease, HSCRP, MDA, Oxidative Stress, Polycystic Ovarian Syndrome, SOD.

Introduction

Polycystic ovary syndrome (PCOS) is a most common endocrine disorder affecting 5–10% of women in reproductive age. In the year 1935, Irvin Stein and Michael Leventhal described about PCOS which was later known by their name as Stein Levinthal syndrome. This condition first manifests with menstural abnormalities (oligomenorrhoea or amenorrhea), hirsutism, insulin resistance (IR) and infertility.[1,2] PCOS is a disorder which affects the various functions in the body like endocrine, metabolic and reproductive functions. PCOS is the most common disorder which causes chronic anovulation leading to infertility in women of reproductive age.[3] CVD, type 2 Diabetes mellitus, endometrial and breast cancer are long term complications seen in PCOS patients but CVD is highly prevalent. The potent CVD risk factors like obesity, diabetes and dyslipidemia, insulin resistance, endothelial dysfunction associated with PCOS might be the cause for high risk of PCOS patients developing CVD.[1,3] Oxidative stress(OS) which plays a key role in the pathogenesis of CVD was documented in obese PCOS women. Oxidative stress may influence not only on cardiovascular system but also on female reproductive system leading to infertility.[4] Oxidative stress also plays a key role in the pathophysiology of PCOS but the exact cause of OS in PCOS is not completely understood. Studies suggests the presence of IR and hyperglycemia seen in PCOS women are profound factors to increase the oxidative stress.[4] Oxidative stress defined as an imbalance between the production of ROS and antioxidant defense system is an important component of cardiometabolic risk seen in PCOS women and is known to independently contribute to endothelial dysfunction.[3] OS along with IR seen in PCOS are associated with endothelial dysfunction which is an early reversible marker of atherosclerosis. Low grade chronic inflammation seen in PCOS women is an independent marker of CVD; the presence of which is characterised by rise in serum crp. This selectively binds to oxidised LDL molecules and gets deposited in atheromatous plaques and contributes in pathogenesis and progression of atheroma due to its pro inflammatory property; all these could together lead to cardiovascular risk in PCOS.[1]

Materials and Method

A cross sectional study was conducted for a period of one year from December 2011 to December 2012 in the Department of Biochemistry of JJM medical college Davangere. A total number of 60 subjects in the age group of 25 to 30years were selected from the Department of OBG of Bapuji hospital Davangere after obtaining approval from institutional ethical committee. Samples were collected from all the subjects after obtaining an informed consent. Clinical history and

physical examination of each subject was carried out. The height and weight of all individuals were measured. Body mass index (BMI) was measured as kg/m^2.

Diagnosis of PCOS was done according to the Rotterdam ESHRE revised consensus 2003.[5] According to it any of the two of the following features should be present to diagnose PCOS:

- chronic oligomennorhoea/ ammennorhoea
- clinical and biochemical evidence of hyperandrogenism
- Polycystic ovaries on ultrasound.

Inclusion Criteria: 60 age and BMI matched subjects within the age group of 25-35 years of which 30 patients with PCOS were taken as cases and 30 normal women were taken as controls. Patients with complaints of any of the two of the following were included:

- Oligomenorrhoea/ amenorrhea
- Inability to conceive
- Hirsutism
- Polycystic ovaries in Ultrasonography.

Subjects included in the study were never on any hormonal contraceptives, aspirin, statins, vitamin supplements or any other significant drug therapy. Age matched 30 healthy women with regular menstrual cycles (28-32days) were included as controls.

Exclusion Criteria: Subjects suffering from any infections and inflammatory conditions, congenital adrenal hyperplasia, hyperprolactinemia, Cushing's syndrome were excluded from the study.

Methods: After overnight fasting for 12 hours, about 6 ml of blood was drawn under aseptic conditions from the selected subjects. From the 6 ml blood, 2 ml was collected in Fluoride-EDTA tube and 4 ml was collected in a plain tube. Plasma for estimation of glucose was obtained by centrifugation of the fluoride EDTA tube and used for the estimation of plasma glucose concentration. The blood in the plain tube was allowed to clot for 30 minutes and was centrifuged at 5000 rpm; the serum obtained was used for measurement of Insulin, MDA, SOD and hs-CRP. Concentration of plasma glucose was estimated by glucose oxidase method using analytical kits from Erba Diagnostics semi-autoanaylzer(CHEM-5 Plus V2, Erba).[6] Human insulin chemiluminescence immunoassay (CLIA) kit from Acculite-Monobind was used for estimation of insulin in serum in Lumax CLIA microplate reader.[7] Homeostasis model assessment method (HOMA-IR) was applied for calculating insulin resistance.[8] Serum levels of MDA was estimated by Nadiger et al method,[9] SOD by Marklund and Marklund method[10] and hscrp –by Immunoturbidometry method using analytical kits from Erba Diagnostics Mannheim semi-autoanaylzer(CHEM-5 Plus V2, Erba Mannheim).[11]

Results

Results were expressed as mean ± SD. Students unpaired t-test is used for comparing the means of two groups. The p value of <0.05 is considered statistically significant and 0.001 as highly significant.

A significant elevation in serum levels of Insulin, IR, MDA, hscrp ($p<0.001$) and significantly decreased serum level of SOD P ($p<0.05$) was seen in PCOS patients as compared to controls (Table 1). The mean fasting plasma glucose in cases and controls is 94.56 ± 18.87 and 84.83 ± 15.28 respectively. The mean serum insulin levels in cases and controls is 23.91±9.30 and 11.44 which is significantly increased. MDA is increased significantly ($p<0.001$) in PCOS as compared to controls. The mean SOD levels in cases and controls are 3.6±1.15 and 12.55±2.28. hscrp levels were significantly increased ($p<0.001$) in cases as compared to controls with mean value of 4.61±2.05 and 0.42±0.21 in cases as compared to controls

Table 1: Baseline characteristics in non-obese women with PCOS and controls

characteristics		Cases	controls	p value
Age (years)	Mean ± SD	25±2.1	26±1.5	0.041
BMI (kg/m^2)	Mean ± SD	23.41±1.0	22.2±2.2	<0.001
Fasting glucose (mg/dl)	Mean ± SD	94.56±18.87	84.83±15.28	0.016
Insulin (µIU/ml)	Mean ± SD	23.91±9.30	11.44	<0.001
IR (HOMA-IR)	Mean ± SD	5.77±3.03	2.10±0.78	<0.001

Table 2: The serum levels MDA, SOD and hscrp in women with PCOS and in normal women

Parameter		Cases	Controls	P value
MDA (nmol/ml)	Mean ± SD	6.47±1.13	2.36±0.63	<0.001
SOD (U/ml)	Mean ± SD	3.69±1.15	12.55±2.28	<0.001
hscrp (mg/L)	Mean ± SD	4.61±2.05	0.42± 0.21	<0.001

Discussion

In our study the mean fasting plasma glucose in cases is significantly increased compared to controls. A significant elevation in serum levels of Insulin, IR, MDA and hscrp ($p<0.001$) and significantly decreased serum level of SOD ($p<0.05$) was seen in cases as compared to controls. Free radicals, capable of independent existence are highly reactive molecular species with one unpaired

electron in the outermost shell. Free radicals persist for very short duration before achieving stability by colliding with another molecule to either abstract or donate an electron. During this process they generate another free radical from the molecule with which they collided. The most damaging among free radicals in the biological system are the oxygen radicals so these free radicals are called reactive oxygen species (ROS). These ROS targets proteins, carbohydrates, nucleic acids which are oxidized to give a diverse array of products. Polyunsaturated fatty acids (PUFA), present in the cell membrane are readily attacked by oxidising radicals, this process is known as lipid peroxidation forming various end products among which malondialdehyde (MDA) is one of the important end products of this process. MDA is one of the most common biomarker to assess the oxidant status, as its levels correlates with the extent of lipid peroxidation. Antioxidant enzymes present in the body help in maintaining the redox balance of cell. The imbalance between oxidants and antioxidants leads to OS in body. There are various antioxidant enzymes in the body but SOD enzyme is thought to be one of the major enzyme that protect the cells from ROS as it converts the superoxide anion radical into hydrogen peroxide.[1,2,12] Findings of our study that is the presence of oxidative stress in lean PCOS women is in agreement with various other studies.[3,13,14,15] Oxidative stress has been implicated as one of the common cause in various pathologies like CVD, neurological diseases, malignancies, diabetes, inflammatory conditions and aging etc. Studies conducted in PCOS patients have demonstrated presence of oxidative stress in these patients, cause of oxidative stress in PCOS is not fully understood and various causes have been postulated like hyperglycemia, insulin resistance and chronic inflammation as the cause for OS in PCOS.[1,2,12] Insulin resistance is seen in PCOS women.[16] OS is increased due to IR as hyperglycemia and higher levels of free fatty acid leads to excess production of ROS. IR, hyperglycemia seen in our study supports the findings. Hyperglycemia also plays a role in inflammation by producing TNF α from MNC. ROS produced from MNC results in cellular damage which activates nuclear factor--κB, a pro inflammatory transcription factor which promotes the transcription of TNF α which is a known mediator of IR.[3,17,18] Studies conducted in lean healthy reproductive age women having hyperglycemia suggest that excess androgen increases the generation of ROS from leukocytes, p47phox gene expression and formation of MDA. Hyperandrogenism is seen in PCOS women, and OS is implicated as the cause for it. The presence of OS in absence of obesity could be due to diet induced OS and hyperandrogenism being the progenitor.[2,3,16,19] A close inter relation exists between OS and chronic inflammation; inflammation induces the formation of ROS, while OS promotes and aggravates inflammation.[1,12] Inflammation is considered to be the key feature of atherosclerotic plaques. The occurrence of atherothrombotic events is due to its strong relationship with systemic inflammatory activity. Hepatocytes produce crp, one of the markers of inflammation under the influence of pro-inflammatory cytokines like Tumor necrosis factor- α (TNF α) and interleukins 1 and 6.[1,2] A powerful predictive relationship exists between elevated CRP production and cardiovascular diseases. The significantly increased serum level of hsCrp in the patients than controls indicates presence of low grade inflammation. Boulmer et al conducted a study to find the hsCrp levels in PCOS patients and the risk associated with it for future CV events and divided the patients into low, moderate and high risk groups having (1, 1-3 mg/l, 3mg/l hscrp levels) respectively. Hence a powerful correlation exists between elevated crp and CVD.[1]

Conclusion

In the present study we found increase in serum levels of MDA, hscrp and decrease in SOD levels which indicates the presence of oxidative stress and inflammation; the risk factors for CVD due to hyperglycemia in non-obese PCOS women. Hence correcting oxidative stress with antioxidants and regular monitoring of glucose levels can reduce the complications.

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