EVALUATION OF INSULIN RESISTANCE AND OXIDATIVE STRESS IN OBESE PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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BACKGROUND : Patient with Polycystic ovary syndrome (PCOS) are known to have high incidence of Insulin Resistance (IR) and oxidative stress and most of the patients are obese. The aim of this study was to evaluate the insulin resistance and oxidative stress in obese PCOS patients.

METHODS : 104 obese patients with PCOS [BMI ≥23 Kg/m^2] and 95 healthy control subjects were included in this study. Plasma sex steroid hormones, fasting insulin, fasting glucose, were measured in both the groups. Oral glucose tolerance test was also performed. Total antioxidant status (TAS), malonyldialdehyde (MDH), protein carbonylation (PC), erythrocyte reduced gluthathione (GSH) and antioxidant enzymes like erythrocyte catalase and erythrocyte glutathione peroxidase (GPx) activity were measured. Insulin resistance was evaluated by using homeostasis model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI) and fasting glucose / fasting insulin ratio (fasting G:I ratio).

RESULTS : Compared with healthy women, those with obese PCOS had significantly elevated HOMA-IR, plasma MDA, protein carbonylation, erythrocyte glutathione peroxidase activity and significantly decreased QUICKI, fasting G:I ratio, plasma TAS, GSH, catalase activity.

CONCLUSION: The result of the present study indicates, high level of insulin resistance and significantly increased oxidative stress were observed in obese PCOS patients when compared with controls.

Key words : Insulin resistance / Oxidative stress / PCOS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common and heterogeneous endocrine disorders among women of reproductive age. The prevalence is estimated at 5-10% (knochenhauer et al 1998, Azziz et al 2004, Goldenbery N et al 2008). It is characterized by mensural irregularities, clinical and/or biochemical hyperandrogenism and hyperinsulinaemia secondary to reduced insulin sensitivity (Homburg, 2003). There is no consensus on the diagnostic criteria and definition of PCOS. According to ESHRE/ASRM (European Society for Human Reproduction and Embrology / American Society for Reproductive Medicine) consensus conference on PCOS held in rotherdam in 2003, PCOS was defined as at least 2 out off three following abnormalities (a) oligo and/or anovulation, (b) clinical and/or biochemical signs of hyperandrogenism (hirsutism and/or acne or increased androgens levels) (c) Detection of polycystic ovaries by ultrasound and presence of 10 or more cyst of 2-10mm in diameter in each ovary and absence of other endocrine conditions such as thyroid disorder, cushing's syndrome, congenital virilizing adrenal hyperplasia or hyperprolactinemia.

The etiology of PCOS is still unknown. However insulin resistance and associated hyperinsulinemia play important roles in the pathogenesis of PCOS (Dunaif A, 1997). IR and hyperinsulinemia appears to interfere with ovarian steriodogenetic defects as well as anovalatory mechanism (Poretsky L P-999). These two factors produce the hyperandrogenism interfering with pituitary ovarian axis, leading to increased LH levels, anovulation, amenorrhea and infertility. PCOS is associated with an increased risk of metabolic complications including impaired glucose tolerance, type 2 diabetes, hypertension, dyslipidemia. Several studies have documented that atleast 30-40% of PCOS patients were obese or over weight and obesity has an aggravating role in PCOS. (Diamantri – Kandarakis et al 1999, Azziz et al 2004)

Oxidative stress means an imbalance between the production of reactive oxygen species and the antioxidant defence system which produces the oxidative damage. Oxidative stress has been implicated in a number of diseases such as cardiovascular disease, neurologic disease, malignancies, renal disease, diabetes, inflammatory problems, skin diseases, aging, respiratory diseases, liver diseases and different types of viral infections (Karinsky N.J. 1992, Betheridge et al, 2000). Oxidative stress also impairs insulin action, which has been demonstrated in type 2 diabetes and this impairment might be due several factors such as membrane fluidity alterations, decreased availability of nitric oxide and increased intracellular calcium content (Caimi et al 2003, Elizabeth H et al 2008). TAS is sensitive to the changes in plasma antioxidant levels and degree of oxidative stress (Garibaldi et al 2001). MDA is a marker of lipid peroxidation and increases in oxidative stress states (Knight JA et al 1987, Batteridge 2000). Recent studies have documented increased oxidative stress in patients with PCOS (Sabuncu T et al 2001, Fenkei et al 2003, Zhang D et al 2008) which may increase the risk of cardiovascular disease in such patients.

Till date there are no studies related to the same in Indian studies. Therefore the aim of this study was to evaluate the insulin resistance and oxidative stress in obese PCOS patients.

MATERIALS AND METHODS

The study was approved by Institute research council board and followed by human ethical committee, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. The written informed consent was obtained from patients and controls.

The diagnosis of PCOS was based on the ESHRE/ASRM consensus conference held in Rotterdam in 2003. Patients with diabetes mellitus, thyroid dysfunction, cushing's syndrome, congenital andrenalhyperplasia, hyper proloctinemia, androgen secreting tumour, renal and liver disorders were excluded from the study by specific laboratory tests. Subjects with medication like ovaluation induction agent, antiandrogens, antidiabetic, antiobesity, hormonal drugs and current or previous use of OC within last 6 month smoking and alcohol intake were also excluded from study.

Subjects

One hundred and four patients with PCOS aged between 20 to 35 years were recruited from the out patient department of Obstetrics and Gynecology, JIPMER, Puducherry, India. The control group consisted of 95 healthy volunteer females with regular menstrual cycles aged between 20 to 35 years.

Body mass index (BMI) and waist to hip ratio (WHR) were calculated. weight and height of the subjects were measured in light clothing without shoes. BMI was calculated as weight by height square (Kg/m^2). Waist circumference was measured at narrowest level between costal margin and the Iliac crest and hip circumference was measured at the widest level over the buttocks with subject was standing and breathing normally. Degree of hirsutism was determined by using Ferryman-Gallwey scoring. A score ≥8 was (Ferriman-Gallwey, 1961) defined as Hirsutism.

After 10-12 hours fasting 7ml of anticoagulant venous blood was collected between 8.00 and 8.30am on the 2^{nd} day of spontaneous or progesterone (Medroxy progesterone acetate-10mg/day for 7 days) induced withdrawal bleeding for estimations of insulin resistance markers, sex steroid hormones and oxidative stress parameters, prolactin, cortisol, thyroid profile, liver function test and renal function test etc.

A standard 75gram glucose tolerance test (OGTT) was performed after 10-12 h fasting between 8.30 and 10.30am. Glucose tolerance state was evaluated using the criteria of American Diabetes Association 2003. Those patients with DM were not included in the study. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the following formula: fasting glucose (mmol/L) x fasting insulin (µu/ml) / 22.5 (Mathews et al 1985). The quantitative insulin sensitivity check index (QUICKI) was calculated according to following formula : 1/[log fasting insulin (µu/ml) + log fasting glucose (mg/dl)] (Katz et al, 2000). Fasting glucose (mg/dl)-Insulin (µIu/ml) ratio also calculated (Parra A et al 1994).

Plasma glucose (Agappe-India) was estimated by glucose oxidase-reroxidase (GOD-POD) method by clinical chemistry autuanalyser. [Bayer Express plus, USA] Plasma Insulin was (Bio line, Belgium) estimated by ELISA Techniques (Lab system-Multiskan Ascent, Finland).

Plasma TAS was measured spectrophotometrically at 593 nm using ferric reducing antioxidant power assay (FRAP). The results were expressed as µmol of FRAP/ml. Plasma MDA levels were estimated by spectrophotometry at 530nm by Yagi (Yagi-1984). The results were expressed µmol/ml, plasma protein carbonylation was estimated by spectrophotometry at 366nm by modification of Levines method by chakroborthy. The results were expressed as nmol/ml. Erythrocyte reduced glutathione (GSH) was estimated by spectrophotometry at 412nm by Beutle et al (Beutle et al 1963). The results were expressed as mg/g Hb. Haemoglobin was estimated with Drabkin reagent (Merk, Bombay, India) for calculation of glutathione assay. RBC-catalase activity was assayed by spectrophotometry at 240nm by Aebi (Aebi 1984). The results were expressed as K/ml. The RBC glutathione peroxidase (GPx) activity was determined by spectrophotometry at 412 by Wendel method (Wendel et al 1984). The results were expressed as U/gmHb.

Plasma levels of LH, FSH, Prolactin (immuno tech, Czech republic) and TSH (BRIT, India) were estimated by immuno radiometric assay (IRMA) (PC-RIA-MAS STRA Tec-Germany), plasma total testosterone, progesterone (immune tech-czech republic), estradiol (RADIM-Italy), T3, T4 (BRIT-India) and cortisol (Diasoria-USA) were estimated by RIA. (PC-RIA-MAS STRA Tec-Germany) and free testosterone, Androstenedione, 17-Hydroxy progesterone (17-OHP), Progesterone, sex hormone binding globulin (SHBG) and dehydroepiandrostenedion sulphate (DHEA-S) (EQUIPAR-Diagnostic, Italy) were measure by ELISA technique (Stat-Fax, 300 CPC-Germany).

STATISTICAL ANALYSIS

Data was expressed as mean ± S.D. For comparison of all quantitative variables between control and PCOS groups, the unpaired t-test was used and association between the parameters were assayed by Pearson correlation analysis. Statistical significance was accepted at $P<0.05$. All statistical analysis were performed using SPSS Program (Chicago, IL).

RESULTS

Mean age, BMI and basal clinical and endocrinological parameters of PCOS patients and control subjects were shown in Table I. Basal Insulin resistance and oxidative stress parameters were shown in Table II.

TABLE I : BASAL CLINICAL AND HORMONAL PARAMETERS IN CONTROL AND PATIENTS WITH OBESE PCOS SUBJECTS

Variables	Controls (n=95)	PCOD (n=104)
Age (Yrs)	27±4	27.33±3.30
BMI (Kg/m^2)	22.08±1.75	27.39±1.45*
W/H ratio	0.82±0.02	0.85±0.01*
F-G score (≥8)	0/75	17/87*
LH (µIu/ml)	5.98±1.03	13.10±7.00*
FSH (µIu/ml)	5.74±1.16	5.27±1.85
Total Testosterone (ng/dl)	36.60±8.15	63.98±16.65*
Free Testosterone (Pg/mg)	2.01±0.62	3.12±0.74
Androstnedione-A4 (ng/dl)	1.47±0.45	3.64±0.87*
Progesterone (ng/dl)	0.45±0.40	0.61±0.39
17-Hydroxy progesterone (ng/dl)	0.53±0.02	0.84±0.18
Estradiol-E2 (Pg/ml)	58.92±17.21	39.03±11.51
Sex hormone binding globulin SHBG-(nmol/L)	62.39±8.35	42.98±11.44*
Dehydro epiandrostenedion sulphate DHEA-S (µg/dl)	173.12±44.72	262.64±72.33*

* $P<0.05$ compared to controls.

Table I shows baseline clinical and endocrinological parameters of control subjects and obese PCOS patients. Mean age between control and obese PCOS groups were similar. Compared with healthy women, those with obese PCOS had increased BMI, WHR ratio and F-G score ($P<0.05$).

Hormonal Parameters :

Compared to control, subjects with obese PCOS exhibited higher level of LH ($P<0.05$), total testosterone ($P<0.05$), and androstenedione ($P<0.05$) and DHEA-S ($P<0.05$), where as decreased levels of sex hormone binding globulin ($P<0.05$).

TABLE II : BASAL INSULIN RESISTANCE AND OXIDATIVE STRESS PARAMETERS IN CONTROL AND PATIENT WITH OBESE PCOS SUBJECTS

Variables	Controls (n=95)	PCOD (n=104)
Fasting glucose (mg/dl)	83.76±6.6	106.64±12.56**
Fasting insulin (µIU/ml)	14.27±2.92	35.61±5.3**
HOMA-IR	2.92±0.53	9.49±2.36**
QUICKI	0.33±0.09	0.28±0.09**
Fasting G:I ratio	6.20±1.79	3.01±0.33**
MDA (µmol/L)	3.20±0.73	4.90±1.63*
PC (µmol/ml)	3.21±0.79	4.95±1.61*
TASC (µmol/ml)	129.16±22.08	96.11±17.82*
Erythrocyte GSH (mg/g Hb)	7.37±7.61	4.59±17.82*
Erythrocyte catalese (K/ml)	23.44±6.71	15.31±4.3*
Erythrocyte GPx (U/g Hb)	25.19±6.13	36.04±7.22*

**P<0.005 compared to controls.
* P<0.05 compared to controls.

Insulin resistance

Table II shows baseline insulin resistance and oxidative stress parameters of control and obese PCOS patients. Insulin resistance markers like fasting glucose, fasting insulin and HOMA-IR ere significantly increased in obese patients than the control subjects (P<0.005) where as QUICKI and fasting G/I ratio were decreased (P<0.005).

Oxidative stress

Women with obese PCOS had significantly higher plasma MDA, Plasma protein corbonylation and increased activity of erythrocyte glutathione peroxidase (P<0.05) than the control subjects. Where as plasma TAS, erythrocyte GSH levels and erythrocyte catalase activity were lowered in obese PCOS than the control (P<0.05).

Correlation Analysis

The correlation between oxidative stress and antioxidant parameters in PCOS cases are shown in table III. Both MDA and protein carbonyl were significantly correlated with TAS, GSH, Catalase and GPx (P<0.01).

TABLE III: CORRELATION BETWEEN OXIDATIVE STRESS AND ANTIOXIDANT PARAMETERS IN PCOD CASES

Variables	MDA		Protein carbonyl	
	r	p	r	p
TAS	- 0.832	< 0.01	- 0.849	< 0.01
GSH (mg/g Hb)	- 0.711	< 0.01	- 0.769	< 0.01
Catalase (K/ml)	0.740	< 0.01	0.832	< 0.01
GPx (U/g Hb)	0.737	< 0.01	0.783	< 0.01

The correlation between oxidative stress and antioxidant parameters insulin resistance in PCOD cases are shown in table IV. MDA, PC, Catalase and GPx were positively correlated with insulin and HOMA-IR and negatively correlated with QUICKI (P<0.01). TAS and GSH were negatively correlated with insulin and HOMAIR and positively correlated with TAS and GSH (P<0.01).

Table 22: Correlation between oxidative stress and insulin resistance in PCOD cases

Variables	Insulin r	HOMA-IR r	QUICKI r
MDA (µmol/L)	0.778*	0.763*	-0.752*
PC (nmol/ml)	0.787*	0.789*	-0.771*
TAS (µmol/ml)	- 0.786*	-0.746*	0.724*
GSH (mg/g Hb)	- 0.782*	-0.794*	0.764*
Catalase (K/ml)	0.685*	0.699*	-0.693*
GPx (U/g Hb)	0.740*	0.707*	-0.691*

* $p < 0.01$

DISCUSSION

Insulin resistance with resultant hyperinsulinaemia is a prominent feature of PCOS, and it is seen both in obese and normal weight woman (Chang et al 1983, Dunaif et al 1989). Moreover, obese women develop a greater degree of insulin resistance as their body mass increases (Rittmaster et al 1993, Naveed Satton BHR, 2009).
Recent studies have-shown that PCOS is not only a gynalcological condition affecting women of reproductive age but also a comprehensive syndrome with a variety of associated metabolic disorders such as insulin resistance, dyslipidaemia (Wild et al 2000). The present study evaluated insulin resistance and more recently emerging risk factors such as oxidative stress.
Although the mechanism leading to the development of PCOS are still not completely understood. However insulin resistance and hyperinsulinaemia may play an important role in pathophysiology of PCOS (Dunaif et al 1989 and 1997). Insulin resistance is associated with obesity.(Azziz et al 2004 and Diamantri-kandarakis 2008). In the present study, insulin resistance, as detected by the markers HOMA-IR higher in obese PCOS than in healthy controls and QUICKIand fating G/I ratio were lowered.
Oxidative stress is an accepted risk factor for the development of CVD (Betteridge, 2000). PCOS is also associated with an increased risk of CVD. In PCOS, oxidative stress in response to hyperglycemia may be capable of directly stimulating hyperandrogenism. This is suggested by the association between plasma testosterone or androstedione and reactive oxygen species (ROS) generation. In vitro studies have shown that the ovarian steroidogenic enzymes responsible for androgen production are stimulated by oxidative stress and inhibited by antioxidants such as statins (Piotrowski et al 2005 and R Zepczynska IJ et al 2005 and Elizabeth H et al 2008).
In our study, plasma TAS levels, erythrocyte GSH levels and erythrocyte catalase activity were significantly lower in obese women with PCOS compared with healthy controls. Higher plasma MDA levels, plasma protein corbonylation, and activity of erythrocyte glutathione reductase were found in patients than controls. These findings implicate the presence of increased oxidative stress in obese patients with PCOS.

In conclusion, in the present study, we have evaluated insulin resistance and oxidative stress and their relationship in obese PCOS patients. Insulin resistance and oxidative stress and accepted risk factors for CVD. The present study confirmed that such risk factors are present more in obese patient with PCOS in our population. Further more we found that increased levels of androgens in obese PCOS patients.

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