EVALUATION OF ENDOCRINE PARAMETERS AND GLUCOSE TOLERANCE STATUS IN OBESE SUBJECTS WITH POLYCYSTIC OVARY SYNDROME

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BACKGROUND: Severe insulin resistance is a key abnormality in obese women with polycystic ovary syndrome (PCOS). The purpose of this study was to evaluate the sex steroid hormone levels and glucose tolerance status in obese PCOS patients.
METHODS: 104 obese patients with PCOS (BMI≥23Kg/m^3) and 95 healthy control subjects were included in this study. Body mass index (BMI), waist-to-hip ratio (WHR), plasma sex steroid hormones, fasting insulin, fasting glucose were measured. Oral glucose tolerance test was also performed according to WHO criteria. Hirsutism score and acne were recorded. 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: plasma LH, LH/FSH ratio, total Testosterone, adrostenedione and dehydroepiandrostenedione sulphate (DHEA-S) were higher where as SHBG was lower in patient with obese PCOS compared to controls ($P<0.05$). Compared with healthy women, those with obese PCOS had significantly elevated fasting insulin, HOMA-IR, and significantly decreased QUICKI and fasting G:I ratio.
CONCLUSION: The results of the present study indicates, elevated androgen levels, high degree of glucose intolerance and insulin resistance in obese PCOS patients when compared with healthy control subjects.

Key words : Insulin resistance / PCOS / Glucose intolerance.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a most common heterogeneous endocrine disorders among women of reproductive age. The prevalence is estimated at 5-10% (knochenhauer et al, 1998, Azziz et al 2004). PCOS is characterized by oligomenorrhea amenorrhea, infertility, hyperandrogenism, insulin resistance, early onset of type 2 diabetes mellitus, dyslipidemia and cardiovascular disease (Franks.S 1995, Carmina E et al 1999, Knochenhauer ES et al 1998). Women with PCOS indicates marked clinical heterogencity; the commonly associated features of hirsutism, acne, poly cystic-appearing ovaries, obesity and acanthosis rigricans are neither uniform nor universal (Acien P et al 1999, Hacihaarefioglou B 2000).

Approximately half of all women with PCOS are over weight or obese (Gambini A et al 2002). Obesity, especially central visceral obesity is present in 35% to 80% of patients with PCOS and has been shown to be an independent predictor of conversion to impaired glucose tolerance or type 2 DM in women with PCOS (Norman RJ et al 2001).

According to ESHRE/ASRM (European Society for Human Reproduction and Embrology/American Society for Reproductive Medicine) consensus conference on PCOS held in Rotterdam in 2003, PCOS was defined as at least 2 out off three following abnormalities (a) oligo and/or an ovulation, (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 remains unclear. However insulin resistance and associated hyperinsulinemia play important roles in the pathogenesis of PCOS (Dunaif A 1997). Insulin Resistance and hyperinsulinemia appears to interfere with ovarian steroidogenetic defects as well as anovulatory mechanisms and involved in the metabolic aspects of PCOS. Insulin resistnace appears to be an integral features of PCOS, independent of other factors (Poretsky T. et al 1999).

Previous studies have reported that altered gonadotrophins, hyperandrogenism, insulin resistance and glucose intolerance in PCOS patients. (Conway et al 1989, Frnaks S 1989, Carmina E et al 1998, ciorardli TP et al 1992, Reri's AN et al 1987, Moghethi et al and Ehrmann DA et al 1997). Till date there are no studies related to the same in indian subjects. Therefore the primary aim of our study was to evaluate the endocrine parameters, glucose tolerance status and insulin resistance 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 andrenal-hyperplasia, hyper prolactinemia, 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 ninety five 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 2nd 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, 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 was also calculated (Parra A et al 1994).
Plasma glucose (Agappe-India) was estimated by glucose oxidase-peroxidase (GOD-POD) method by clinical chemistry autuanalyser. [Bayer Express plus, USA] Plasma Insulin (Bio-line, Belgium) was estimated by ELISA Techniques (Lab system-Multiskan Ascent, Finland).
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. 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 1, Basal Insulin resistance markers shown in Table II, 2 hours oral glucose tolerance test (OGTT) and impaired fasting glucose (IFG) and impaired glucose tolerance test (IGT) were shown in Table 2 and 4 respectively.

TABLE 1: Basal Clinical and Hormonal Parameters in Control and Patients With Obese Pcos Subjects

Variables	Controls (n=95)	PCOD (n=104)
Age (years)	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	17/104*
Acne	0/75	11/104*
LH (µIu/ml)	5.98±1.03	13.10±7.00*
FSH (µIu/ml)	5.74±1.16	4.61±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 endocinological 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 F-G score and acne (P<0.05).

Hormonal Parameters

Compared to control, subjects with obese PCOS exhibited higher levels 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 2 : BASAL INSULIN RESISTANCE 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**

**P<0.005 compared to controls.

Insulin resistance

Table 2 shows baseline insulin resistance parameters of control and obese PCOS patients. Insulin resistance markers like, fasting insulin and HOMA-IR were significantly increased in obese PCOS patients than the control subjects (P<0.005), where as QUICKI and fasting G/I ratio were decreased (P<0.005).

Glucose Tolerance

Table 3 shows 2 hours OGTT values in controls and obese PCOS women and Table 4 shows impaired fasting glucose and impaired glucose tolerance status.

Table 3: 2 Hours Oral Glucose Tolerance Test in Controls and Obese Pcos Cases

Variables	Control (n=95)		PCOD (n = 104)	
	Fasting	2h OGTT	Fasting	2h OGTT
Glucose (mg/dl)	83.76±6.67	96.28±6.82	106.64±12.56**	153.32±10.13**
Insulin (µIU/ml)	14.27±2.92	79.69±8.40	35.61±5.31**	167.13±11.24**

**P<0.005 compared to controls.

Fasting Glucose, Insulin and 2 hours OGTT glucose and insulin were significantly higher in obese PCOS women than control subjects (P<0.005). Patients with obese PCOS had 43.3% (45/104) impaired fasting glucose and 66.5% impaired glucose tolerance (69/104).

Table 4: Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) In Controls and Obese Pcos Cases

Variables	Control (n=95)	PCOS (n=104)	Percentage
Impaired fasting glucose (IFG)	0/95	45/104*	43.3
Impaired glucose tolerance (IGT)	0/95	69/104*	66.5

DISCUSSION:

The current study was undertaken to evaluate sex steroid hormones and glucose tolerance status in obese PCOS patients in Indian population. Although mechanism leading to the development of PCOS are still not completely understood, it become apparent that insulin resistance and hyperinsulinaemia may play vital roles in the pathophysiology of PCOS (Dunaif A et al 1989, Dunaif A 1997). Insulin resistance is associated with obesity. In the present study we have selected only obese PCOS patients, since majority of the patients attending the infertility clinics were obese in our populations compared with other populations. (Diamantri-Kanderatis et al 1999, Azzi et al 2004, Gambireri et al 2006, Hounteya Sinha-2007). In our study women with PCOS were 6.7% (7/104) overweight and 93.3% (97/104) obese compared with age matched controls. Previous studies shown that approximately 50% women with PCOS were obese (Frank S 1995 and Apridonidze T et al 2005). Obesity and PCOS are common yet complex disorders reflecting an influences of environemtnal and genetic factors (Legro RS et al 1999). Other studies have found that the prevalence of obesity in PCOS populations, although high, in variable among large series (Balen AN et al 1995).

Regardless of the presence of obesity, many are also insulin resistance. Insulin resistance with resultant hyperinsulinaemia is a prominent features of PCOS, and it is seen in obese and normal weight women (Chang et al 1983, Dunaif A et al 1989 and Naveed Saffar et al 2009). Moreover, obese women develop a greater degree insulin resistance as their body mass increases (Rittmaster et al 1993). In our study obese PCOS patients were found to be more insulin resistance than the age matched control subjects. This was evident from our findings that both fasting insulin and markers of insulin resistance like HOMA-IR, QUICKI and G/I ratio were significantly altered in PCOS cases. This finding was supported by similar studies done by several investigators (Ferriman and Purdie 1999, Carey et al 1993, Naman et al 1993, Pontiroli et al 2001, Legro et al 2002, Sir-Petermann et al 2002, Yieldi Z et al 2003 and Ehrman et al 2005).

According to Dunaif and coworkers, obese PCOS women had significantly increased glucose levels during an oral glucose tolerance test when compared with age and weight matched control women. Recent studies have sugges that the prevalence rate of glucose intolerance is a high as 40% in women with PCOS by WHO criteria (Ehrmann et al 1999, Legro et al 1999). Another study was reported that the prevalence of glucose intolerance has been 30-40% among both lean and obese adolescents with PCOS (Palmert et al 2002). Several groups have assessed glucose tolerance among PCOS women and the overall risk of developing Type 2 diabetes was found to be increased three to seven times (Punaif et al 1987, Dahlgren et al 1992, Ehrmann et al 1999, Legro et al 1999, Wild et al 2000). They found that 31-35% had impaired glucose tolerenace and 7.5-10% had type 2 diabetics.

Furthermore, the onset of glucose intolerance in PCOS women serum to accur at an early age, typically in the third to fourth decade of life, which is earlier than in the normal population (Harris et al 1987, Kind and Rewens 1993, Ehrmann et al 1999, Legro et al 1999). Risk factors associated with glucose intolerance in PCOS are obesity, age. BMI, waist to hip ratio family history of Type 2 Diabetes and androgen concentration which are indentical in general population. (Harris MI et al 1996, Harins MI 1997, Legro et al 1999, Ehrmann et al 1999, Gambineri et al 2004).

Suprinsingly in our study showed that women with obese PCOS 43.3% (45/104) imparied fasting glucose and 66.5% (69/104) impaired glucose tolerance. Therefore our results suggesting that Indian women with obese PCOS had higher prevalence of glucose intolerance (66.5%) than the age matched healthy women. In addition, result also showed that prevalence of glucose intolerance in obese PCOS more when compared with other population.

A number findings suggest, that hyperinsulinaemia may play a central role in the development of hyperandrogenism (Dunaif A et al 1989 and Dunaif A 1997, Azziz et al 2004 and Gambinen et al 2006). Some studies have reported that increased hyperandrogenism in obese compared with nonobese (Bulent O et al 2008).

Hyperandrogenism is a key feature of PCOS with elevations of ovarian androgens, testosterone and androstendione (Cormina 2002), sex hormone binding globulin SHBG is generally low in PCOS, primarily due to obesity, leading to higher free testosterone levels. Women with PCOS, as evidenced by higher total testosterone and/or free testosterone or free and rogen index, but comparable androstenedione and DHEAS levels (Holte J et al 1994, Grulet H et al 1993 and Conway GS 1992). The present study of in our populations, plasma levels of LH, LH/FSH ratio, total testosterone, androstenedione and dehydroepiandrostenedione sulphate were higher in patient with obese PCOS compared to healthy controls, where as sex hormone binding globulin significantly lower. We found that, obese PCOS patients had 16.3% (17/104) hirsutism and 10.6% (11/104) acne.

In conclusion, in the result of present study support that high prevalence of obesity in women with PCOS in our populations and had high degree of biochemical and clinical hyperandrogenism, increased prevalence of glucose intolerance and insulin resistance. Furthermore based upon this evidence that high prevalence of obesity, high incidence of glucose intolerance and high degree of insulin resistant in obese PCOS patients may develop type 2 diabetes and cardiovascular disease later on. Therefore, obese PCOS patients need to be monitored for DM and CVD and preventive measured to be taken for Indian populations.

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