

Original Research Article

The plasma nitric oxide and homocysteine levels and their association with insulin resistance in South Indian women with polycystic ovary syndrome

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ABSTRACT

Background: Women with polycystic ovary syndrome (PCOS) exhibit features of the metabolic syndrome apart from low-grade chronic inflammation and endothelial dysfunction and may be at increased risk for cardiovascular disease (CVD). The Nitric oxide (NO) and Homocysteine (Hcy) are important plasma markers of endothelial dysfunction, an early marker of atherosclerosis. There are no Indian studies on NO and Hcy levels in women with PCOS and their association with Insulin Resistance (IR). Therefore the present study is to estimate plasma levels of NO and Hcy in south Indian women with PCOS and association with insulin resistance.

Methods: 104 women with PCOS and 95 healthy age matched control subject were enrolled in the study. Standard physical methods and Chemiluminescent Immunoassay technique were employed for estimation of Anthropometric parameter and plasma sex hormones respectively. Fasting insulin, glucose, NO and Hcy were measured by standard methods. Insulin resistance was evaluated by using Homeostasis Model Assessment for Insulin Resistance (HOMA-IR)

Results: Women with PCOS had significantly higher insulin resistance ($P < 0.01$), Hcy ($p < 0.05$) and lower NO levels ($P < 0.05$), IR was positively correlated with Hcy ($r = 0.610$, $p < 0.01$) and negatively correlated with NO ($r = -0.285$; $p < 0.01$)

Conclusions: Our data revealed that South Indian women with PCOS had elevated IR and homocysteine and lowered NO levels.

Keywords: Endothelial dysfunction, Homocysteine, Insulin resistance, Nitric oxide, Polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a most common complex, metabolic, endocrine, multifaced and highly heterogeneous disorder of women of reproductive age with unknown etiology and prevalence being 5-10 % worldwide.¹ It is one of the most common causes of oligo/anovulation, biochemical or clinical hyperandrogenism and polycystic ovaries and is

associated with metabolic features including insulin resistance (a key pathophysiological component), impaired fibrinolysis and endothelial dysfunction.^{2,3} Although prospective controlled data on cardiovascular disease (CVD) morbidity and mortality in PCOS patients are lacking.

The endothelium plays a crucial role in regulating vascular function and dysfunction of endothelial cells is probably the earliest event in the process of lesion

formation and atherosclerosis.⁴ Endothelial function can be investigated by measuring circulating markers like nitric oxide and Hcy, which may be useful prognostic and/or therapeutic monitoring tool for patients with CVD risk factors.⁵

A recent meta-analysis of 21 studies showed that endothelial dysfunction was evident in women with PCOS even if they were young and non-obese: Nitric oxide, free radical gas molecules, has been shown to be involved in numerous physiological and pathological processes.⁶ The bio availability of NO is reduced due to increased level of superoxide radicals, which transforms NO to peroxynitrite due to increased oxidative stress. NO is continuously released from endothelial cells to keep vessels dilated and shows endothelial integrity. Free oxygen radicals may also interact with NO release and decreased NO will also demonstrate endothelial dysfunction.⁸ There is less limited number of studies, indicates that the levels of NO in women with PCOS is contradictory.⁹

Homocysteine is an amino acid formed by the conversion of methionine to cysteine. Elevated plasma Hcy levels are considered to be an independent risk factor cardiovascular disease because of increased oxidative stress in the vascular endothelium and activation of platelet aggregation.¹⁰ Several studies have shown that elevated plasma concentration of Hcy in both lean and obese with PCOS versus control.¹¹ Several but not all studies have found insulin resistance to be the most important predictor of increased Hcy.¹² Recent study showed that elevated plasma Hcy levels is present in both obese and non-obese women with PCOS than controls.¹³

In view of high prevalence of obesity in India owing to westernization, urbanization & mechanization and evidence suggesting a pathogenetic role of obesity in the development of PCOS and related infertility.¹⁴ Therefore, the aim of the present study was to investigate the plasma markers of endothelial dysfunction, NO and Hcy in south Indian women with PCOS and relationship with insulin resistance.

METHODS

One hundred and four patients with PCOS aged between 20 to 35 years were recruited from outpatient department of Obstetrics and Gynaecology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER) Puducherry, India. The control group consisted of ninety five healthy volunteer females with regular menstrual cycles aged between 20 to 35 years. The diagnosis of PCOS was made according to the ESHRE/ASRM-sponsored PCOS consensus workshop group guidelines.

The study was approved by Institute Research Council Board and followed by Human Ethical Committee,

JIPMER, Puducherry, India. The written informed consent was obtained from patients and controls.

Patients with diabetes mellitus, thyroid dysfunctions, Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, androgen secreting tumor, renal and liver dysfunction were excluded from the study by specific laboratory tests. Subjects with medication like ovulation induction agents, anti-androgens, antidiabetic, antiobesity, hormonal drugs and current or previous use of OC within last 6 months, smoking and alcohol intake were also excluded from the study.

All 104 PCOS patients and 95 healthy control underwent full physical examination and anthropometric measurements including weight, height and waist and hip circumferences and were asked to complete a general questionnaire. Weight was measured with the subjects wearing right clothing without shoes, and height was measured using a stadiometer. Body Mass Index (BMI) was calculated by using the formula: weight (Kg)/height(meters). Waist circumferences (WC) was measured with the patients standing at a point mid way between lower costal margin, and illia crest in the mid-axillary line. Hip circumferences was measured at the widest point over the buttocks. The presence and extent of hirsutism was quantified using the Ferrinman-Gallwey (F-G) score.

After overnight fasting, venous blood sample was obtained between 08.00 am and 08.30 am, on the 2nd day of spontaneous progesterone (metoxy progesterone acetate 10 mg/day for 7 days) induced withdrawal bleeding for (to carry out the study during the follicular phase we had to induce the menstruation in PCOS women because they were having irregular menstrual cycles) estimation of hormones, IR marker indices, NO and Homocysteine. The plasma glucose was determined by glucose oxidase-peroxidase, end point method using a commercial kit (Agape diagnostic, India) using clinical chemistry Auto analyzer (Beckman Coulter AU680, Japan).

Serum LH, FSH, insulin were determined by two-site sandwich immunoassay chemiluminiscence method by Siemens Advia Centaur CP analyzer. Serum Testosterone, Androstenedione, Progesterone, Estradiol, DHEAS and Hcy were analyzed by competitive immunoassay of Chemiluminiscence method by Siemens Advia Centaur CP analyzer. The SHBG was measured by competitive immunoassay of Chemiluminiscence method by chemi analyzer. The concentration of NO was measured as nitrite/nitrate by spectrophotometric method using Griess reagent. IR was determined by Homeostasis Model Assessment for insulin resistance (HOMA-IR) = Fasting glucose (mg/dl)x fasting insulin (μ IU/ml)/405.

All the statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 16.0 for Microsoft windows. All data were presented as mean+standard

deviation. The paired 't' test was used to compare the parameters of control and cases. Pearson's correlation test was used to assess the association between the parameters in PCOS patients. Statistical significance was considered as $P < 0.05$.

RESULTS

The clinical and hormonal data of women with PCOS and controls are shown in Table 1. There was no significant difference in age but BMI, F-G score, LH, total Testosterone androstenedione were significantly higher in women with PCOS than controls ($p < 0.01$) and similarly WHR, LH/FSH ratio and progesterone, DHEAS were also significantly higher in women with PCOS than healthy controls ($p < 0.05$). On the other hand, patients with PCOS had significantly lower levels of FSH, estradiol and SHBG than controls ($P < 0.05$).

Table 1: Clinical features, hormonal profiles in women with polycystic ovary syndrome and healthy controls.

| Variables | Control (n=95) | PCOS (n=104) |
|----------------------------|----------------|----------------|
| Age (years) | 27±4 | 27.33±3.30 NS |
| Weight (Kg) | 57±12 | 68±4** |
| Height (Cm) | 1.59±0.05 | 1.58±3.38 NS |
| BMI (Kg / m ²) | 22.08±1.75 | 27.39±1.45** |
| Waist circumference (Cm) | 75±4 | 89±3* |
| Hip circumference (Cm) | 91±5 | 104±4* |
| Waist-hip ratio | 0.82±0.02 | 0.85±0.01* |
| F-G Score (>8) | 3.8±1.2 | 10.2±1.6** |
| LH (μIU/ml) | 5.98±1.03 | 13.10±7.00** |
| FSH (μIU/ml) | 5.74±1.16 | 4.61±1.85* |
| LH / FSH | 1.05±0.11 | 2.83±0.76* |
| TT (ng/dl) | 36.60±8.15 | 63.98±16.65** |
| Androstenedione (ng/dl) | 1.47±0.45 | 3.64±0.87** |
| Progesterone (ng/dl) | 0.45±0.40 | 0.61±0.39* |
| Estradiol (pg/ml) | 58.92±17.21 | 39.03±11.51* |
| SHBG (nmol/L) | 62.39±8.35 | 42.98±11.44* |
| DHEAS (μg/dl) | 173.12±44.72 | 262.64±72.33** |

Values are shown in mean ± standard deviation; * $p < 0.05$ and ** $p < 0.01$, NS = Not significant.

The insulin resistance indices, NO and Hcy levels in women with PCOS and control are listed in Table 2. The fasting plasma glucose and plasma Hcy levels were significantly increased in PCOS than controls group ($p < 0.05$). The fasting serum insulin and HOMA-IR were also significantly increased in PCOS patients than control ($p < 0.01$). The QUICKI and NO concentration were significantly lowered in PCOS patients than in control groups ($p < 0.05$).

Table 2: Insulin resistance indices, NO and Hcy levels in women with PCOS and healthy control.

| Variables | Control (n=95) | PCOS (n=104) |
|--------------------------|----------------|---------------|
| Fasting glucose (mg/dl) | 83.76±6.67 | 106.64±12.56* |
| Fasting insulin (μIU/ml) | 14.27±2.92 | 35.61±5.31** |
| HOMA-IR | 2.92±0.53 | 9.49±2.36** |
| QUICKI | 0.33±0.09 | 0.28±0.09* |
| NO (μmol/L) | 2.5±0.8 | 4.8±1.36* |
| Hcy (μmol/L) | 7.11±2.41 | 12.43±2.6* |

Values are shown in mean±standard deviation; * $p < 0.05$ and ** $p < 0.01$ compared to controls.

The correlation between insulin resistance (HOMA-IR), NO and Hcy are listed in Table 3. Insulin resistance was negatively correlated with NO ($r = -0.280$ $p < 0.05$) and positively correlated with Hcy ($r = 0.610$, $p < 0.01$).

Table 3: The correlation between insulin resistance, (HOMA-IR), nitric oxide and homocysteine in PCOS patients.

| Variables | HOMA - IR | |
|--------------------------|-----------|---------|
| | r value | p value |
| NO (μ mol/L) | -0.285 * | <0.05 |
| Homocysteine (μ mol / L) | 0.610** | <0.01 |

DISCUSSION

PCOS is associated with clustering of cardiovascular risk factors in both modifiable and non-modifiable. Although prospective studies and clinical trials with large samples sizes and long-term follow-up are lacking.¹⁵ Endothelial dysfunction, an early marker of vascular disease, is a state linked reduced nitric oxide (NO) bioavailability and increased oxidative stress. In addition, women with PCOS display signs of endothelial dysfunction and data on endothelial dysfunction in patients with PCOS are poor and conflicting.¹⁶

NO contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation and leukocyte adhesion to endothelium. In our study, NO levels measured as nitrite (NO^-) and nitrate (NO^-) were decreased in PCOS patients than in control group. Our findings are also consistent with those of Ahmed M. Mohamadin et al, Mine Yavuz Taslipnar et al and on the other hand, Nacul et al, Kuscu et al, Erdagon et al did not observe significant differences in terms of NO in their studies between PCOS patients and healthy controls.¹⁷⁻²¹ However Karabulut AB et al showed increased NO levels in PCOS group when compared to healthy control.²²

In present study NO negatively correlated with insulin resistance. No adequate studies have been carried out till

date on the association between NO and IR in PCOS patients. However there is only one study by Nacul et al, who reported that a significant negative correlation between NO and IR in PCOS patients. Our data revealed that PCOS is a condition associated with an increased vascular risk, although further studies are needed to clarify the role of NO in PCOS. Several mechanisms may be involved in the development of endothelial dysfunction such as, in the arterial wall; IR is associated with reduced synthesis and release of NO, enhanced inactivation of NO after its release from endothelial cells and enhanced synthesis of vasoconstriction agents.²³

In the present study, women with PCOS had significantly higher Hcy levels than controls, which is similar to reports by some other investigators.²⁴⁻³⁰ Mancini et al, have shown that no significant difference in Hcy levels in PCOS women and controls.³¹ Hcy has a well-known role in cardiovascular morbidity and mortality and it has primary atherogenic and prothrombotic properties. In recent studies, plasma Hcy concentrations were found to be elevated in PCOS women, suggesting that an alteration in Hcy metabolism might play a role in the increased cardiovascular risk associated with PCOS.³² High Hcy levels are considered to be an independent risk factor for cardiovascular disease because of the increased oxidative stress in the vascular endothelium and activation of platelet aggregation and leads to endothelial dysfunction. Our data showed that a significant positive correlation between Hcy and insulin resistance in PCOS patient, in agreement with vrbikova et al and Tayebe H et al.³³ It has been reported that insulin resistance and hyperhomocysteinemia contributed to the long term complications of PCOS. However, further longitudinal studies are needed to investigate their possibility.¹¹

CONCLUSION

In the present study, insulin resistance and resultant hyperinsulinemia are cardinal features of PCOS women. The plasma concentration of NO was significantly lower and whereas Hcy concentration was higher in South Indian women with PCOS compared to healthy women. These results showed that elevated insulin resistance and plasma, Hcy levels and lower levels of NO which are the possible risk factor for CVD in PCOS patients. Further prospective studies with larger numbers of patients and control groups are necessary to confirm our results.

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