

# Insulin Resistance and Homocysteine Levels in Patients with Polycystic Ovarian Syndrome

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## ABSTRACT

**Objectives:** To assess association in between insulin resistance and homocysteine levels in patients with polycystic ovarian syndrome (PCOS).

**Methods:** A case control study.

**Setting:** Hospital based.

**Patients:** Forty females diagnosed with PCOS were matched with 40 normal healthy controls.

**Intervention:** Blood samples for insulin, lipid profile, fasting glucose and homocysteine measurements.

**Results:** Our study suggests that PCOS patients have insulin resistance, dyslipidemia and hyperhomocysteinemia when compared with normal healthy controls.

**Conclusion:** To determine the short-term implications of reproductive performance and long-term implications regarding cardiovascular complications associated with PCOS the assessment of insulin resistance, lipid profile along with homocysteine levels in patients of PCOS is warranted.

**Keywords:** Polycystic ovarian syndrome, Insulin resistance, Homocysteine.

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**Conflict of interest:** None declared

## INTRODUCTION

Homocysteine (HCY) is a thiol containing amino acid produced by the intracellular demethylation of methionine. Hyperhomocysteinemia (HHCY) has been described as an independent risk factor for cardiovascular disease. HHCY either by elicitation of oxidative stress, systemic inflammation and/or endothelial dysfunction is known to promote insulin resistance and  $\beta$ -cell dysfunction.<sup>1</sup> At present, polycystic ovarian syndrome (PCOS) which is characterized by hyperandrogenism, chronic anovulatory cycles, and oligomenorrhea is considered not only a disease that influences fertility but also a pluri metabolic syndrome. The endocrine disturbances typical of PCOS are said to be connected to insulin resistance and impaired glucose tolerance. Also, data has shown an increased prevalence of cluster of cardiovascular risk factors, such as type 2 diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease in PCOS women.<sup>2</sup> This metabolic syndrome of insulin resistance in PCOS can hence be another well-described cluster of risk factors along with HCY.

However, various studies<sup>3-5</sup> in literature have shown conflicting reports regarding insulin resistance and homocysteine levels in patients with PCOS. Hence, with this in mind the present study was designed to evaluate homocysteine levels and the relationship in between homocysteine and insulin resistance in female patients with PCOS.

## MATERIALS AND METHODS

A case control study approved by the Institutional Ethics Committee was carried out at NKP Salve Institute of Medical Sciences, Nagpur. Forty women with PCOS (mean age: 28.27  $\pm$  3.36 years) were matched with 40 normal healthy controls (mean age: 27.4  $\pm$  2.73 years). PCOS was defined when at least two of the following features were present, i.e. oligo or anovulation (fewer than six menstrual periods in the preceding year), clinical (Ferriman-Gallwey score >8) and/or biochemical signs of hyperandrogenism and polycystic ovaries. Biochemical criteria included abnormal LH: FSH ratio (>2) and or elevated testosterone levels. Ultrasound criteria used for diagnosis of polycystic ovaries include: Presence of 12 or more follicles in each ovary measuring 2 to 9 mm in diameter, and/or increased ovarian volume (>10 ml). All the females had normal thyroid, renal, hepatic functions and their prolactin level was normal. All females with pregnancy, current or previous use of oral contraceptives, vitamins, antiandrogens, antidiabetics, statins, lipid lowering agents, glucocorticoids or hormonal drugs, cigarette smoking, chronic alcohol consumption, hypertension, cardiovascular disease, diabetes mellitus, any chronic disorder were excluded from the study. The normal healthy control group comprised of females with regular menses and ultrasonographically normal ovaries. Among 5 ml fasting blood sample was collected from the subjects and analyzed. Serum glucose was measured by using glucose oxidaseperoxidase kit method. Plasma insulin levels were measured by chemiluminescent enzyme immunoassay. The blood samples for homocysteine after collection were immediately centrifuged and stored at  $-20^{\circ}\text{C}$ . The samples were analyzed by microplate enzyme immunoassay kit method of Biorad Laboratories. Estimation of insulin resistance by HOMA score was calculated with the formulae: Fasting serum insulin ( $\mu\text{U/ml}$ )  $\times$  fasting plasma glucose (mmol/l)/22.5, or the logarithmic transformation of the HOMA index ( $\log_{10}\text{HOMA}$ ) i.e. QUIKI.<sup>6</sup>

Serum total cholesterol, triglyceride and HDL-cholesterol were measured by an enzymatic kit. LDL-C was calculated according to the formulae of Friedwalds:  $\text{VLDL-C} = \text{Triglyceride}/5$  and  $\text{LDL-C} = \text{Total cholesterol} - (\text{VLDL-C} + \text{HDL-C})$ .

Statistical significance of difference was estimated using Students 't' test and correlation between variables was studied by using Pearson's correlation coefficient test.

## RESULTS

As depicted in Table 1, the patients with PCOS have insulin resistance as indicated by the levels of plasma insulin, HOMA index and QUIKI. Also there is a highly significant difference in the levels of lipid profile ( $p < 0.001$ ) as indicated by total cholesterol, triglycerides, LDL-cholesterol and VLDL—cholesterol in patients with PCOS when compared with normal healthy controls. Also, serum homocysteine levels are significantly increased in females with PCOS ( $p < 0.001$ ) when compared with normal healthy controls. In our study there is a highly significant correlation in between the two variables, i.e HOMA index and homocysteine in patients with PCOS ( $-0.45$ ,  $p < 0.001$ ). Also there is a significant correlation when fasting glucose ( $0.69$ ,  $p < 0.001$ ), fasting insulin ( $-0.62$ ,  $p < 0.001$ ), total cholesterol ( $0.78$ ,  $p < 0.001$ ), triglycerides ( $0.45$ ,  $p < 0.001$ ), LDL- cholesterol ( $0.44$ ,  $p < 0.001$ ), VLDL—cholesterol ( $0.63$ ,  $p < 0.001$ ) are compared with serum homocysteine levels in patients with PCOS.

There is also a significant positive relationship when fasting glucose levels are compared with total cholesterol ( $0.54$ ,  $p < 0.001$ ), triglycerides ( $0.49$ ,  $p < 0.001$ ), LDL ( $0.48$ ,  $p < 0.001$ ) and VLDL ( $0.41$ ,  $p < 0.001$ ). Moreover, the values of fasting insulin are inversely correlated when compared with total cholesterol ( $-0.52$ ,  $p < 0.001$ ), triglycerides ( $-0.48$ ,  $p < 0.001$ ), LDL ( $-0.47$ ,  $p < 0.001$ ) and VLDL ( $-0.34$ ,  $p < 0.01$ ).

## DISCUSSION

Hyperinsulinemia is known to be characteristic feature of women with PCOS, independent of obesity. Our study suggests that the PCOS patients as judged by the HOMA index have insulin resistance and dyslipidemia. This is in correlation with the study of Tosi F,<sup>2</sup> Jia Huang<sup>7</sup> and Rosolova H.<sup>8</sup> The presence of a post-binding defect in the insulin receptor mediated signal transduction along with a reduction in the GLUT4 glucose transporters in the PCOS adipocytes may be a contributing factor. Other factors include: Decrease in the insulin-stimulated

receptor tyrosine phosphorylation and hyperglycemia-induced insulin resistance mediated by the protein kinase C-induced serine phosphorylation of the insulin receptor in PCOS fibroblasts and a significant alteration in the insulin receptor serine/threonine phosphorylation in the skeletal muscle.<sup>9</sup>

Homocysteine is an essential intermediate in the transfer of activated methyl cycle which is responsible for the regulation of gene expression. Plasma levels of insulin influence HCY metabolism through effects on glomerular filtration or by influencing activity of pivotal enzymes in HCY metabolism, as methyltetrahydrofolate reductase (MTHFR) and cystathionine  $\beta$  synthase. The increase in the levels of HCY in patients with PCOS in our study is similar to the that of Mohan and Vishnu Priya.<sup>10</sup> The abnormal rise in levels of HCY appears to contribute to the vasculature disease by numerous mechanisms which include: Endothelial dysfunction, impairment of endothelium dependent vasodilatation, induction of oxidative stress on the endothelial cells, elevated plasminogen activator inhibitor-1, decreasing the bioavailability of nitric oxide, promoting the growth and proliferation of nitric oxide, interference with the clotting factors and oxidation of low density lipoproteins.<sup>4,6,11,12</sup>

HCY levels are influenced by a number of variables which include smoking, renal function, vitamin B status and enzyme dysfunctional states. All the patients enrolled in our study were nonsmokers, in good general health and none had hypertension or edema. Genetic factors play an important role in the metabolic pathway of HCY synthesis. The enzymatic defects due to genetic mutations induce a significant increase in HCY concentration. Since, the frequency of mutation of MTHFR is 5 to 10% in total unselected population, hence MTHFR status being responsible for the difference between HCY in PCOS and controls seems unlikely and hence MTHFR, an enzyme involved in the folate-dependent remethylation of HCY to methionine were not screened in our study. This is based on the observation of Schacter.<sup>6</sup> As per our knowledge, there is no data available in literature regarding the correlation of serum HCY levels and C677T MTHFR polymorphism in women with PCOS. Orio<sup>13</sup> demonstrated that in women without thermolabile MTHFR enzyme insulin resistance seems to play a role in the HCY metabolism and this thermolabile MTHFR enzyme influences serum HCY concentrations only in healthy women, where as it is not related to HCY levels in PCOS.

In our study the positive correlation in between insulin resistance and homocysteine in patients of PCOS is similar to that of Schacter et al.<sup>6</sup> Talbott et al<sup>14</sup> in their study suggested an evidence for the association between PCOS and premature carotid atherosclerosis in middle aged women. Tanrikulu-Kilic et al<sup>15</sup> and Rosolova<sup>8</sup> however, suggested that plasma homocysteine concentrations are not related to insulin resistance and/or metabolic abnormalities in premenopausal women and healthy subjects respectively.

Our findings suggest that insulin resistance, hyperinsulinemia and HCY are associated in patients with PCOS regardless of their body weight.

The primary treatment in patients with PCOS is targeted to either the menstrual irregularity, androgen excess and infertility.

**Table 1:** Clinical and biochemical parameters in controls and PCOS patients

	Control (n = 40)	PCOS (n = 40)
Age (years)	27.4 $\pm$ 2.73	28.27 $\pm$ 3.36
BMI (kg/m <sup>2</sup> )	21.13 $\pm$ 2.34	24.83 $\pm$ 2.87
Waist circumference (cm)	74.32 $\pm$ 2.8	95.02 $\pm$ 11.16
Ferriman-Gallwey score	4.02 $\pm$ 1.38	10.15 $\pm$ 1.27
Fasting glucose (mg%)	83.32 $\pm$ 5.6	92.22 $\pm$ 13.41*
Fasting glucose (mmol/l)	4.62 $\pm$ 0.31	5.11 $\pm$ 0.74*
Fasting insulin ( $\mu$ U/ml)	5.42 $\pm$ 1.246	9.03 $\pm$ 1.97
HOMA	1.11 $\pm$ 0.27	2.005 $\pm$ 0.27
QUIKI	2.12 $\pm$ 0.31	1.51 $\pm$ 0.08*
Total cholesterol (mg/dl)	163 $\pm$ 12.08	185.8 $\pm$ 21.13*
Triglyceride (mg/dl)	59.1 $\pm$ 11.11	67.62 $\pm$ 10.51*
HDL cholesterol (mg/dl)	50.43 $\pm$ 7.5	49.85 $\pm$ 8.87
LDL cholesterol (mg/dl)	11.82 $\pm$ 2.22	13.51 $\pm$ 2.09*
VLDL cholesterol (mg/dl)	53.5 $\pm$ 15.9	68.33 $\pm$ 16.31*
Homocysteine ( $\mu$ mol/l)	6.64 $\pm$ 0.94	11.04 $\pm$ 4.52*

\* $p < 0.001$ , when PCOS patients compared with normal healthy controls

In case the symptoms are treated these females may seek no further treatment. Since, in our study there is a positive correlation when fasting glucose levels are compared with lipid profile and homocysteine levels in PCOS patients these patients are at a risk of cardiovascular or cerebrovascular disease. Hence, assessment of further clinical and laboratory investigations like insulin, lipid and HCY may have important diagnostic and treatment implications. This is to determine the short-term implications of reproductive performance and long-term regarding cardiovascular complications associated with insulin resistant PCOS. Thus, given the substantial risk associated with HHCY and the fact that HCY levels can be lowered with non-toxic vitamin therapy, the screening of patients at high risk for cardiovascular disease is warranted in patients with PCOS.

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