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Original Article

# Hyperhomocysteinemia, insulin resistance and body mass index in Iranian young women with polycystic ovary syndrome

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ABSTRACT

*Objectives:* Polycystic ovary syndrome (PCOS) is a common disease among young women that causes hyperinsulinemia. Hyperhomocysteinemia is shown to be correlated positively with the hyperinsulinemia. This study aimed to evaluate serum homocysteine (Hcy) level and its correlation with other serum metabolic amongst over weight, obese and non-obese PCOS women.

*Methods:* Sixty women with PCOS and 20 healthy subjects were studied. Hormonal assays, lipid profile, Hcy and fasting insulin levels, insulin resistance (IR) indices (HOMA and QUICKI) determinations and ultrasound evaluation were performed.

*Results:* Mean of BMI was significantly higher in PCOS subjects as compared with the controls ($29.95 \pm 4.91$ vs $26.09 \pm 4.80$ kg/m$^2$; $p = 0.005$). The mean fasting insulin levels were also significantly higher ($17.80 \pm 8.02$ vs $11.55 \pm 6.81$ μU/dl; $p = 0.003$) where we found no difference in fasting glucose concentrations between groups. IR indices in PCOS women were significantly higher than control group ($p < 0.01$). We observed significantly higher mean serum Hcy levels in PCOS patients ($11.68 \pm 2.92$ vs $9.31 \pm 2.45$ μmol/l; $p = 0.047$). When patients were stratified by BMI, the Hcy concentration was not significantly difference in both obese and normal-weight PCOS women. Fasting insulin concentrations and IR indices were significantly higher in obese PCOS patients as opposed to normal-weight PCOS women. IR indices showed increasing trends with BMI, but not Hcy levels.

*Conclusions:* Our study shows the tendency toward hyperhomocysteinaemia, hyperinsulinemia and higher BMI in PCOS patients. These factors are critical predictors of PCOS; however, BMI and IR are an independent risk factor to increase plasma Hcy levels in PCOS women.



## 1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine system disorder among women in which levels of the sex hormones are disrupted [1]. It is a major cause of ovulatory problems, hyperandrogenism, infertility and increased abortion rate [2]. Recent investigations have also revealed the other potential complications among PCOS patients including hypertension, high cholesterol, anxiety and depression, as well as sleep apnea, endometrial cancer, heart attack, diabetes and breast cancer [1,3]. Furthermore, obesity, hyperinsulinemia and insulin resistance have been also reported in many PCOS women [1,4,5].

Homocysteine (Hcy) is a sulfur amino acid synthesised by the breakdown of methionine and may undergo transsulfuration to cysteine and cystathionine [1]. Numerous studies have indicated increased levels of serum Hcy in PCOS subjects [1,6]. Elevated serum Hcy level is demonstrated to be a risk factor for cardiovascular disease and type 2 diabetic mellitus in PCOS patients [7,8]. Hcy concentration is also influenced by vitamin $B_{12}$ (vit$B_{12}$) and folate levels.

In spite of several studies, the real mechanism in which Hcy is increased in PCOS patients is not well-understood. A lot of factors affect serum Hcy levels including age, gender, sex-steroid hormones, insulin resistance (IR), body mass index (BMI), and glucose tolerance and chronic inflammation [1]. Increased insulin levels have been considered as a modulating factor of Hcy in which insulin inhibits hepatic cystathionine β-synthase activity that increases serum homocysteine [1]. Although several lines of studies considered the relationship between serum Hcy levels, body weight and insulin resistance, this probable correlation is still debatable. For

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example, Henning et al., and Sheu et al., found elevated serum Hcy in patients with hypertension and insulin resistance [9,10]. Conversely, Sills et al., and Kilic-Okman et al., didn't observe a correlation between PCOS and Hcy levels in PCOS patients [11,12]. Therefore, in this research we aimed to investigate this controversial issue to explore the relationship between serum Hcy with several parameters such as body weight and insulin resistance in women with and without PCOS.

## 2. Materials and methods

### 2.1. Subjects

This cross sectional, case control study was conducted at PCOS Clinic of Fatemeh-Zahra infertility Center in Babol, Iran. After signing an informed consent, a total of 80 women aged from 18 to 35 subjects were enrolled which included 60 diagnosed PCOS patients according to Rotterdam European Society of Human Reproduction and Embryology Consensus Meeting 2003 guideline [13] and 20 healthy on PCOS women were taken as controls. The ethics committee of the Faculty of Babol University of Medical Sciences approved the study protocol. Diagnosis of PCOS was based on ultrasound, clinical and biochemical criteria. Ultrasound criteria were used to detect polycystic ovaries, defined as the presence of at least 12 follicles of 2–9 mm diameter, and/or increased ovarian volume (>10 ml) [14]. Clinical criteria included amenorrhea or oligomenorrhea (a cycle length >35 days or six periods per year). Patients who were diagnosed as PCOS also had some traits symptoms of hirsutism with Ferriman and Gallway score ($\geqslant$8), acne, alopecia, according to Ferriman and Gallway [15]. Biochemical criteria included elevated menstrual LH/FSH ratio (>2) or elevated serum testosterone (>2.8) nmol/l and or biochemical signs of hyperandrogenism including: increased circulating level of total or free testosterone, or dehydroepiandrosterone sulfate (DHEAS), and the other causes of hyperandrogenism had been excluded. The patients with the following conditions were also excluded from the study: pregnancy, hyperprolactinaemia, thyroid dysfunction, hypertension, gastrectomy, medications for treatment of cerebrovascular and coronary heart diseases, folate antagonist medication (*i.e.* methotrexate), phenytoin and carbamazepine medications, cigarette smoking, chronic alcohol consumption, contraceptive pills and anti-obesity, strenuous physical activity. Patients who had used hormonal drugs or folic acid and vit$B_{12}$ supplements within the previous 3 months were excluded from the study. Patients have also no history of treatment with the drugs that affect plasma Hcy levels.

### 2.2. Laboratory analysis

BMI was calculated by standard formula: [weight/height$^2$; (kg/m$^2$)]. Waist circumference to hip circumference ratio was reported as WHR in Table 1. Insulin resistance was determined by static fasting glucose and serum insulin levels, HOMA-IR (homeostatic assessment of insulin resistance) and QUICKI (quantitative insulin sensitivity check index) were calculated according to the following formula: HOMA-IR = [fasting insulin (μIU/ml) × fasting glucose (mg/dl)]/405 and QUICKI = [(1/(log fasting insulin (μIU/ml) + log fasting glucose (mg/dl)] indexes [16]. Insulin resistance was defined as HOMA-IR >2.5, abnormal fasting insulin (>20 mIU/l) and glucose/insulin ratio (<0.25) [17]. All hormonal and biochemical experiments were performed at the pathobiology lab of Pastur clinic in Babol, Iran. Blood samples (~10 ml) were collected after an overnight fast for at least 12 h from each patient. All samples were immediately cooled; serum and plasma samples were separated within 1 h and stored at −20 °C until assayed.

**Table 1**
Comparison of clinical and biochemical parameters in PCOS and control groups.

| Parameters | Control (n = 20) | PCOS (n = 60) | P-value |
|---|---|---|---|
| Age (years) | 23.73 ± 3.85 | 23.65 ± 5.08 | 0.946 |
| BMI (kg/m$^2$) | 26.09 ± 4.80 | 29.95 ± 4.91 | 0.005 |
| Waist/hip ratio | 0.88 ± 0.23 | 0.86 ± 0.14 | 0.587 |
| Ferriman-Gallwey score | 4.78 ± 5.42 | 10.68 ± 8.01 | 0.001 |
| FSH (mIU/ml) | 6.44 ± 2.40 | 6.22 ± 2.01 | 0.699 |
| LH (mIU/ml) | 6.86 ± 3.51 | 8.93 ± 6.18 | 0.075 |
| LH/FSH ratio | 1.07 ± 0.62 | 1.41 ± 0.86 | 0.073 |
| Free-testosterone (pg/ml) | 2.67 ± 1.61 | 2.65 ± 1.23 | 0.967 |
| Testosterone (ng/ml) | 0.60 ± 0.24 | 0.89 ± 1.20 | 0.083 |
| 17-OH-progesterone (ng/ml) | 0.97 ± 0.49 | 1.03 ± 0.72 | 0.692 |
| DHEAS (μg/dl)) | 149.76 ± 78.45 | 177.30 ± 90.10 | 0.207 |
| SHBG (nmol/L) | 46.07 ± 30.42 | 35.09 ± 28.38 | 0.153 |
| Prolactin (mIU/L) | 384.67 ± 226.08 | 302.97 ± 173.99 | 0.162 |
| Triglyceride (mg/dl) | 128.89 ± 42.22 | 142.98 ± 76.77 | 0.314 |
| TSH (mIU/ml) | 2.95 ± 2.29 | 2.31 ± 1.14 | 0.111 |
| Total cholesterol (mg/dl) | 187.26 ± 25.71 | 186.20 ± 31.52 | 0.788 |
| LDL-cholesterol (mg/dl) | 106.63 ± 22.56 | 103.61 ± 25.32 | 0.626 |
| HDL-cholesterol (mg/dl) | 52.78 ± 12.31 | 52.73 ± 12.64 | 0.986 |
| Fasting insulin (μU/dl) | 11.55 ± 6.81 | 17.80 ± 8.02 | 0.003 |
| Fasting glucose (μU/dl) | 85.63 ± 7.04 | 89.13 ± 10.12 | 0.099 |
| G/I ratio | 10.34 ± 6.22 | 6.80 ± 5.71 | 0.036 |
| HOMA-IR > 2.5 | 2.49 ± 1.63 | 3.91 ± 1.86 | 0.003 |
| QUIKI < 0.34 | 0.34 ± 0.03 | 0.32 ± 0.026 | 0.007 |
| VitB12 (pg/ml) | 415.13 ± 145.38 | 392.82 ± 137.31 | 0.559 |
| Folate (ng/ml) | 15.09 ± 5.60 | 16.81 ± 6.52 | 0.272 |
| Hcy (μmol/l) | 9.31 ± 2.45 | 11.68 ± 2.92 | 0.046 |

* $p < 0.05$ is considered as significant.

### 2.3. Biochemical analysis

Biochemical analysis including fasting blood sugar (FBS), cholesterol, triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were measured with Azmoon kit (Teif Azmoon Pars Co; Tehran, Iran) by the spectrophotometric method (Hitachi 911 Chemistry Analyzer, Germany). Serum Hcy level was determined using Axis-Shield Homocysteine Enzyme Immunoassay (EIA). The Hcy level $\geqslant$11 μmol/L was considered to be high. The serum vitB12 (pg/mL) and folic acid (ng/ml) levels were measured using Roche kit by electrochemiluminescence assay (ECL). Serum insulin (μlU/ml) was analyzed using immunoradiometric assay kit (BI-Insulin IRMA; Bio-Rad, Marnes-la Coquette, France).

### 2.4. Hormonal analysis

Serum 17-OH-Progestrone level was measured using radioimmunoassay (RIA) method. The serum levels of other hormones including prolactin, testosterone, dehydroepiandrosterone sulfate (DHEAS), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were evaluated with chemiluminescence immuno assay kit (CLIA). Thyroid stimulating hormone (TSH) was determined with Immunoradiometric assay method. Sex hormone binding globulin (SHBGL) was measured by electrochemiluminescence assay kit (ECL). Free testosterone was analyzed using ELISA Kit.

### 2.5. Statistically analysis

Mean standard (mean ± S.D.) of all parameters was analyzed by descriptive statistic. Data were analyzed by Statistical Package for Social Sciences (SPSS-19) version. An independent *t*-test was considered to compare the scores of each of the measures and some of the parameters data between the two groups. Pearson's correlation coefficients were used to calculate correlation between paired data sets. Significance of correlation and the relative contribution of each variable were calculated. A p-value $\leqslant$ 0.05 was considered statistically significant.

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