Turkish Journal of Endocrinology and Metabolism, (2005) 1 : 23-28 **ORIGINAL ARTICLE**

The Plasma Homocysteine Concentrations and Relationship with Insulin Resistance in Young Women with Polycystic Ovary Syndrome

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Women with polycystic ovary syndrome (PCOS) have several cardiovascular disease risk factors including hyperinsulinemia. Hyperhomocysteinemia is a recognized risk factor for atherosclerosis and has recently to be correlated positively with the hyperinsulinemia. We examined the relationship between plasma homocysteine levels and insulin resistance in patients with PCOS. Forty women with PCOS and 35 healthy subjects were studied. Hormonal assays, lipid pofile, homocysteine and fasting insulin levels, insulin resistance indices like HOMA and QUICKI determinations and ultrasound evaluation were performed in all subjects. The mean fasting insulin levels were significantly higher in women with PCOS than control women (12.02 ± 7.6 vs 6.08 ± 2.09 µU/ml) where as no difference in fasting glucose concentrations was observed between groups. Insulin resistance indices (HOMA and QUICKI) were significantly different between PCOS and control group (p<0.001). We found significantly higher mean plasma homocysteine concentrations in patients with PCOS as compared with controls (11.5 ± 2.71 vs 9.4 ± 1.8 µmol/L, p=0.002). When patients were stratified by body mass index (BMI), the homocysteine concentration were significantly higher in both obese and normal-weight women with PCOS than control women (11.65 ± 2.3, 11.15 ± 1.9, 9.4 ± 1.8 µmol/L, respectively). Fasting insulin concentrations and and insulin resistance indices were significantly different in obese PCOS patients as opposed to normal-weight women with PCOS although both obese and normal-weight PCOS patients were more insulin resistant than normal healthy volunteers. As a result, insulin resistance in women with PCOS is associated with elevated plasma homocysteine, regardless of body weight and together with other risk factors like dyslipidemia or hyperinsulinemia, elevated homocysteine levels may contribute to the risk of cardiovascular disease in women with PCOS.

Keywords: Poloycystic ovary syndrome, homocysteine, insulin resistance

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age and is estimated to affect 5-10 % of the population (1). Prominent features of the syndrome include hirsutism, menstrual dysfunction, infertility, elevated androgen levels and insulin resistance (1,2). Women with PCOS have a clustering of cardiovascular risk factors, such as obesity, lipid abnormalities, impaired glucose tolerance and hypertension. Recent data have shown not only an increased prevelance of cardiovascular disease (CVD) (3-5) but also higher cardiovascular morbidity in women with PCOS (6).

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In recent years homocysteine , which is an amino acid containing thiol, has been described as an independent risk factor for CVD (7-9). Plasma homocysteine levels have been shown to correlate with blood pressure (10), body mass index and insulin resistance (11,12). Since hypertension, obesity and hyperinsulinemia are frequently encountered features of PCOS, it seems logical to hypothesize that elevated homocysteine levels could be another feature of PCOS and this feature may contribute to increased prevalance of CVD in women with PCOS. This study was designed to evaluate homocysteine concentrations and the relationship between homocysteine and insulin resistance in normal weight and obese women with PCOS and healthy control group.

Material and Methods

Forty women affected by PCOS (mean age: 24.86 ± 4.7 years) were enrolled in our study. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of the study. PCOS was defined when at least two of the following three features were present after the exclusion of other etiologies. These features were; oligo- or anovulation (fewer than six menstrual periods in the preceding year), clinical (Ferriman-Gallwey score > 8) (13) and/or biochemical signs of hyperandrogenism and polycystic ovaries. Biochemical criteria included an abnormal LH:FSH ratio (> 2) and/or elevated testosterone levels . Ultrasound criteria used for diagnosis of polycystic ovaries (PCO) are the following: Presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume (>10 mL). All women had normal thyroid, renal and hepatic functions. Their prolactin levels were within normal limits. Exclusion criteria for all subjects included pregnancy, current or previous use (within 6 months) of oral contraceptives, vitamins, antiandrogens, anti-diabetics, statins, glucocorticoids or other hormonal drugs, cigarette smoking, chronic alcohol consumption, coffee consumption more than 2 cups/day, blood pressure of ≥ 130/85 mmHg or treated hypertension, known CVD and diabetes mellitus. During the testing period, all subjects were asked to keep their normal diet and not perform any sporting activity. An overnight dexamethazone supression test (1mg) and follicular phase serum 17-OH progesterone determination were performed in order to exclude Cushing's syndrome and late-onset congenital adrenal hyperplasia. The control group consisted of 35 healthy women (mean age: 24.3 ± 4.1 years) with regular menses and ultrasonographically normal ovaries. Their clinical, biochemical and hormonal profiles were within normal limits. Same exclusion criteria as patient group were used for control group.

All blood samples were obtained in the morning between 08.00 h and 09.00 h after a 3 day, 300 g carbohydrate diet, an overnight fasting and during early follicular phase. During the same visit all subjects underwent anthropometric measurements and transvaginal ultrasonography. The serum concentrations of FSH, LH, testosterone, prolactin, SHBG and DHEAS were measured by chemiluminescent enzyme immunoassay (Immulite 2000 Diagnostic Products Corporation LA, CA) Serum glucose was measured by using glucokinase technique. Lipid analysis in fasting serum was performed for all patients. The lipid profile included measurement of the levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride. These parameters were measured by commercial enzymatic methods (Aeroset automated analyzer, Abbott). LDL-cholesterol was calculated by using Friedewald's formula.

Plasma insulin levels were measured by chemiluinescent enzyme immunoassay (Immulite 1000 Analyser) with interassay and intra-assay cofficients of variation (CV) did not exceed 6.4%. Serum vit B12 and folic acid levels were measured by chemiluminescent enzyme immunoassay (Immulite 2000 Diagnostic Products LA, CA). Reference ranges for vit B12 and folic acid were 193-982 pg/ml and 3-17 ng/ml, respectively. Blood samples for homocysteine measurement were collected, immediately placed on ice and centrifugated at 4°C. Plasma was seperated within 30 min and stored at -70°C. The plasma homoysteine concentration was measured by chemiluinescent enzyme immunoassay (Immulite 2000 Diagnosic Products, LA, CA). Normal reference ranges of our laboratory were 5-12 µmol/L. Interassay and intra-assay CV 7.4%.

Insulin resistance (IR) was determined by a number of different methods including fasting insulin, the homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI). The estimate of insulin resistance by HOMA score was calculated with the formula: fasting serum insulin (µU/ml) x fasting plasma glucose (mmol/L) / 22.5 (14). QUICKI is derived by calculating the inverse of sum of logarithmi-ally expressed values of fasting insulin and glucose (15).

Statistical analysis: The statistical Package for the Social Sciences (SPSS version 11.5 for Windows) was used for statistical analysis. Results were expressed as mean ± SD. The characteristics of distribution were tested with Kolmogorov-Smirnof test. A highly skewed variables were analysed after logarithmic transformation. Spearman rank correlations were used for these variables. The Mann-Whitney U test was used for variables with

persisting skewed distribution after log transformation. Differences betwen means were analyzed by Student's unpaired *t*-test using two-tailed tests for significance. P<0.05 was considered statistically significant. Analysis of correlations between parameters was performed by using Pearson's bivariate correlation coefficient.

Results

The overall clinical and hormonal results of patients with PCOS and control subjects are shown in table I. The groups were similar for age, but body mass index (BMI) was significantly higher in PCOS cases as compared with the controls (28.05 ± 6.26 vs 22.2 ± 2.97 kg/m²). The Ferriman-Gallwey score was significantly higher in PCOS cases than in the controls, as well as LH, testosterone and androstenedione levels. The mean fasting insulin levels were significantly higher in women with PCOS than control women (12.02 ± 7.6 vs 6.08 ± 2.09 μU/ml) where as no difference in fasting glucose concentrations was observed between groups. Insulin resistance indices (HOMA and QUICKI) were significantly different between PCOS and control group. No significant difference were detected in serum vit B12 and folate levels between PCOS and control groups. The mean serum levels of total cholesterol and LDL-cholesterol were comparable however, the mean triglyceride concentration was significantly higher and HDL-cholesterol concentration was significantly lower in the PCOS group. In addition to marked differences in hormonal parameters, insulin levels and lipid profiles between PCOS cases and controls, we found evidence of significantly higher homocysteine levels in PCOS patients as compared with the control subjects (11.5 ± 2.71 vs 9.4 ± 1.8 μmol/L).

According to their BMI, the PCOS patients were divided into 2 subgroups. 18 patients out of 40 (45 %) had BMI <25 kg/m² and 22 patients (55 %) has BMI ≥ 25 kg/m². BMI, homocysteine, insulin concentrations and insulin resistance indices of the normal-weight and obese women with PCOS as well as the control group are shown in table II. Homocysteine levels were significantly higher in both obese and normal-weight women with PCOS than control women. Fasting insulin concentrations and and insulin resistance indices were significantly different in obese PCOS patients as opposed to normal-weight women with PCOS although both obese and normal-weight PCOS patients were more insulin resistant than normal healthy volunteers.

Correlation between clinical or biochemical criteria and homocysteine was examined on paired-data basis. Fasting insulin levels were significantly correlated with homocysteine concentrations (p<0.05).

Table 1. Clinical and hormonal parameters of the patient and control groups

	PCOS (n=40)	Controls (n=35)	P value
Age (years)	24.86 ± 4.7	25.4 ± 4.07	NS
BMI (kg/m²)	28.05 ± 6.26	22.2 ± 2.97	<0.001
Waist circumference (cm)	90.16 ± 16.81	74.57 ± 5.32	<0.001
Ferriman-Gallwey score	11.2 ± 1.9	4.8 ± 1.1	<0.001
LH (IU/L)	7.93 ± 5.81	3.89 ± 2.8	<0.01
FSH (IU/L)	4.89 ± 2.03	3.1 ± 1.51	NS
Testosterone (ng/dl)	96.8 ± 67.3	65.5 ± 41.2	<0.01
Androstenedione (ng/ml)	4.08 ± 2.3	2.89 ± 1.1	<0.01
SHBG (nmol/L)	30.7 ± 9.4	58.44 ± 21.3	<0.05
Fasting glucose (mg/dl)	89.4 ± 9.2	83.14 ± 7.9	NS
Fasting insulin (μU/ml)	12.2 ± 7.6	6.08 ± 2.09	<0.001
HOMA	3.01 ± 1.9	1.24 ± 0.4	<0.001
QUICKI	0.335 ± 0.05	0.375 ± 0.005	<0.001
Total cholesterol (mg/dl)	165.2 ± 38.4	160.4 ± 41.3	NS
Triglyceride (mg/dl)	121 ± 99.4	88.6 ± 36.7	NS
HDL-cholesterol (mg/dl)	45.5 ± 13.3	53.6 ± 15.4	<0.05
LDL- cholesterol (mg/dl)	95.36 ± 26.6	101.8 ± 23.5	NS
Vit B12 (pg/ml)	352.14 ± 184.11	373.2 ± 117.6	NS
Folic acid (ng/ml)	8.9 ± 3.45	9.6 ± 2.7	NS
Homocysteine (μmol/L)	11.5 ± 2.71	9.4 ± 1.8	P=0.002

NS: non-significant

Table 2. BMI, homocysteine, insulin concentrations and insulin resistance indices of the normal-weight and obese women with PCOS and the control group.

	Obese PCOS (n=22)	Normal-weight PCOS (n=18)	Controls (n=35)
BMI (kg/m²)	32.2 ± 3.4*	22.37 ± 1.36	22.2 ± 2.97
Homocysteine (μmol/L)	11.65 ± 2.3**	11.15 ± 1.9^	9.4 ± 1.8
Fasting insulin (μU/ml)	15.4 ± 6.4**	8.4 ± 3.4^	6.08 ± 2.09
HOMA	3.7 ± 2.1**	1.9 ± 0.7^	1.24 ± 0.4
QUICKI	0.32 ± 0.2**	0.35 ± 0.02^	0.375 ± 0.005

* p<0.05 obese vs normal-weight PCOS and controls
** p<0.05 obese PCOS vs controls
^ p<0.05 normal-weight PCOS vs controls

Discussion

Recent researches have shown that PCOS is not only the most common reproductive disorder but also a plurimetabolic syndrome (16). Thus women with PCOS have an increased risk of developing type 2 diabetes mellitus, hypertension, dyslipidemia and early-onset cardiovascular disease (17-21). Insulin resistance and resultant hyperinsulinemia are cardinal features of PCOS (19,22). Recently, research has focused on systemic and local effects of insulin resistance in women with PCOS. Hyperinsulinemia in the general population may have various deleterious metabolic effects, including causing an increase of plasma homocysteine levels (11,12,23). Plasma levels of insulin seem to influence homocysteine metabolism through effects on glomerular filtration or by influencing activity of some important enzymes in homocysteine metabolism like methyltetrahydrofolate reductase (MTHFR) and hepatic cystathione β synthase (CBS). Presently plasma homocysteine levels are widely accepted as an independent risk factor for cardiovascular disease (7-9). Homocysteine has been reported to promote atherosclerosis by inducing endothelial dysfunction through limited bioavailability of nitric oxide and altered blood vessel elasticity (24); enhancing the activation of coagulation system and increasing the platelet adhesiveness (25). Plasma homocysteine levels are influenced by a number of variables including smoking, coffee comsumption, renal function, vitB12 and folate status and some drugs (oral contraceptives, vitamins, metformin, etc.) which were taken into consideration for both patient and control group in this study. We examined the association between plasma homocysteine levels and insulin resistance in our specific population of patients with PCOS. Yarali et al. (26) looked at the cardiac diastolic dysfunction of PCOS patients as detected by echocardiography and have shown significantly higher plasma homocysteine concentrations in both lean and obese PCOS patients than control group and this was related to insulin resistance. Schachter et al. (27) also reported that insulin resistance and hyperinsulinemia in patients with PCOS was associated with elevated plasma homocysteine levels regardless of body weight. Another study has shown that PCOS patients had elevated plasma homocysteine levels independent from their BMI (28). Wijeyaratne et al found elevated homocysteine levels in patients with PCOS and this was correlated significantly with fasting insulin (29). However, as mentioned before, plasma homocysteine levels are influenced by a number of variables and neither of these studies were examined these variables in patient and control groups. Sills et al did not find any association between the finding of PCO and plasma homocysteine levels (30). This study differentiated between patients on the basis of ultrasound morphology only, not incorporating other components of the syndrome. Some other studies did not find any association between the finding of PCO, plasma homocysteine and insulin levels (31,32).

VitB12 and folate levels were examined and no significant differences were found between PCOS and controls similar to other studies (26,31). Dyslipidemia may contribute to increased cardiovascular risk in patients with PCOS. Similar with the results of previous studies (Wild, 1995; Meirow et al., 1996; Legro et al., 1999, Sayın et al., 2003) we found an unfovarable lipid profile in patients with PCOS, manifested by increased triglyceride concentration and decrease HDL-cholesterol concentrations.

In conclusion, in present study we have demonstrated that mean serum homocysteine concentrations are increased in women with PCOS. Since most of the variables that influence the homocysteine concentration were taken into account, hyperinsulinemia might be responsible for the higher homocysteine levels in these patients. Together with other risk factors like dyslipidemia or hyperinsulinemia, elevated homocysteine levels may contribute to the risk of cardiovascular disease in women with PCOS.

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