Correlation between Homocysteine and Insulin Resistance in women with Polycystic Ovarian Syndrome Referring to AL-Yarmook Teaching Hospital

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Abstract:

Background:

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders among females in reproductive age. Women with PCOS have several cardiovascular disease risk factors. Hyperinsulinemia and insulin resistance (IR) which is a known key factor in the development of type 2 diabetes (T2DM) may associate PCOS. Homocysteine, a sulfur-containing amino acid, is toxic to vascular endothelium, leads to early atherosclerosis. PCOS said to be associated with high plasma homocysteine.

Objective:

To determine the correlation between insulin resistance and homocysteine in PCOS patients.

Methods:

Patients diagnosed as PCO according to Androgen Excess and PCOS Society/2009 criteria were grouped into two groups: those with BMI less than 30kg/m2 and those with BMI 30kg/m2 and more. Blood was drawn for biochemical and hormonal assay. Information tabulated, means and percentages used, and the results were analyzed.

Results: Homocysteine levels were significantly higher (P value < 0.05) in PCOS group (12.28±1.89 µmol/l) as compared to control (9.51±1.92 µmol/l).HOMA-IR was significantly higher (P value < 0.05) in PCOS (4.15±2.54) as compared to control (1.69±0.54) .There was no correlation between IR and homocysteine within PCOS group.

Conclusions:

Polycystic ovarian patients have higher levels of homocysteine and IR compared to control but there is no correlation between IR and homocysteine within PCOS group.

Key words: Polycystic ovarian syndrome, homocysteine, insulin resistance.

Introduction:

Polycystic ovarian syndrome is the most common endocrinological disorder amongst reproductive age women. The pathophysiology of PCOS is complex in that it causes a spectrum of manifestation [1-3].The exact etiology of PCOS remains unknown, but hyperandrogenism was thought to be the main underlying factor. The phenotypic manifestations of the syndrome vary from patient to patient and the Rotterdam diagnostic criteria for PCOS[4, 5, 6] are based on the clinical identification of at least two of the three defined criteria which includes: - Oligo/anovulation, clinical and /or biochemical evidence of hyperandrogenaemia ,ultrasonography finding of the polycystic ovaries.

Polycystic ovary syndrome is associated with a variety of comorbidities such as diabetes, hypertension, dyslipidemia, cardiovascular events and malignancies that manifest at young age [7].

It has been shown that PCOS may be associated with insulin resistance and the insulin secretion was disturbed to some extent [8].The hyperinsulinemic state in PCOS patients appears to play a central role in disease development and is considered to be the cause rather than the result of hyperandrogenism [9].

Homocysteine is an intermediate substance in methionine metabolism. Elevated levels of plasma homocysteine have been implicated as a significant risk factor for cardiovascular disease, pre-eclampcia, and recurrent pregnancy loss [10]. Homocysteine has a well-known role in cardiovascular morbidity and mortality through its primary atherogenic and prothrombotic properties. It was postulated that homocysteine levels are higher in patients with PCOS [11].

It seems logical to hypothesize that elevated homocysteine levels could be another feature of PCOS and this features may contribute to long term complications of PCOS [12].This study was designed to evaluate homocysteine concentration and its relationship with insulin resistance in women with PCOS.

Patients and Methods:

Fifty-five women with PCOS considered as a patients group, attending the infertility center in AL-Yarmook Teaching Hospital were enrolled in this study their mean age was (26.95±5.5 year).The diagnosis of PCOS was made according to the Rotterdam European Society of Human Reproduction and Embryology (ESHRE) [6].The study includes women who were presented with features of PCOS with BMI more than 25kg/m^2 while the exclusion factors were: (1) any subject had BMI less than 25 kg/m^2 (2) those taken metformin treatment (3) any woman receive treatment for ovulation induction (4) diabetic patients (5) women with pituitary or thyroid problems.

The control group consists of 40 healthy women with regular menses and ultrasonographically normal ovaries. Their age and BMI were matched with PCOS group.

Blood sample were collected in early follicular phase (cycle day2-5) for hormonal evaluation follicular stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) total testosterone and prolactin. The serum concentration of FSH, LH, and total testosterone were examined by MINIVIDUS device while TSH and prolactin hormones were examined by GAMMA method.

Pelvic ultrasound was taken for each woman to examine the presence or absence of polycystic ovary.

For each subject in this study, 5 ml blood sample was collected after 12 hour fasting and centrifuged for serum separation in two tubes one for the lipid profile and fasting blood sugar (FBS) measurement and the other undergo freezing about(-20 C^0) for insulin and

homocysteine analysis which were measured by ELIZA method. IR was calculated by Homeostatic Model Assessment of IR (HOMA-IR) and depends on equation below:

$$HOMA - IR = \frac{\text{Blood glucose (mg/dl)} \times \text{serum insulin (}\mu\text{U}}{405}$$

[13].

The cut-off value of homocysteine level was 13 µmol/l, any measures higher than this level were considered as hyperhomocysteinemia [14].

Statistical Analysis:

All data were coded and computerized analysis using the Statistical Package for Social Sciences (SPSS 17) program and Microsoft office (2010). Independent-sample T-test was utilized to compare difference between parametric data group as mean ± standard deviation. Significant relationship between homocysteine and IR or relationships between other parameter were evaluated by spearman correlation coefficient. P-value of 0.05 and lower was considered as significant.

Results:

All the subjects included in this study were matched for age, BMI, waist circumference, hip circumference, and waist/hip ratio. There was no significant difference between the mean of PCOS group and the control (P>0.05).

Table (1) shows no significant difference in their demographic and anthropometric parameters (P>0.05).

Table (1): Comparison between PCOS group and control group in age and anthropometrics.

Parameter	Control group n=40 Mean+SD	PCOS group n=55 Mean+SD	P value
Age (year)	28.7±5.29	26.95±5.5	0.1203
BMI (kg/m²)	32.6913.72	32.6314.27	0.9468
WC (cm)	95.08±8.49	94.71±9.68	0.8455
HC (cm)	112.03±7.64	113.18±8.26	0.4833
WC/HC Ratio	0.84±0.04	0.83±0.06	0.2808

- PCOS= Polycystic ovary syndrome.
-BMI= body mass index.
-WC.=Waist circumference.
-HC.= Hip circumference.

As shown in table (2) serum FSH, LH were significantly differed, being higher concerning LH and lower FSH in PCOS as compared to control. LH/FSH ratio was significantly higher (P < 0.05) in PCOS group than that in control group. There was no significant difference (P>0.05) in the Testosterone levels between them.

Table (2): Comparison of hormonal analysis between PCOS group and control group.

Parameter	Control group n=40 Mean±SD	PCOS group n=55 Mean±SD	P value
FSH (mIU/ml)	7.15±1.59	5.8±1.8	0.0002
LH (mIU/ml)	5.45±2.16	9.5±5.9	0.0001
LH/FSH ratio	0.76±0.28	1.75±1.22	0.0001
Total Testosterone (ng/ml)	0.5±0.23	0.49±0.3	0.7573

- FSH– Follicular stimulating hormone.

- LH– Luteinizing hormone.

Fasting blood sugar, serum insulin, HOMA-IR, and plasma homocysteine were significantly higher in PCOS group than in the control group as shown in table (3).

Table (3): Comparison between PCOS group and control group related to fasting blood sugar, insulin, HOMA IR, and homocysteine.

Parameter	Control N=40 Mean±SD	PCOS N=55 Mean±SD	P value
FBS (mmol/l)	4.73±0.62	5.11±0.6	0.0036
S. Insulin(µu/ml)	8.02±2.19	17.64±9.3	<0.0001
HOMA-IR	1.69±0.54	4.15±2.54	<0.0001
S. Homocysteine (µmol/l)	9.51±1.92	12.84±6.98	0.0013

FBS= Fasting blood sugar

There is no obvious correlation between IR and homocysteine in PCOS group (Figure 1).

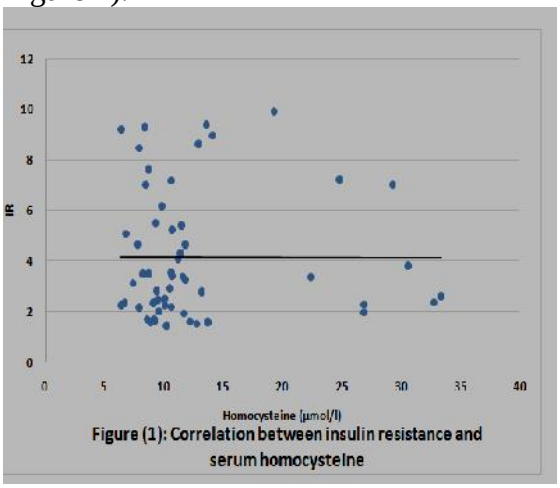

Figure (1): Correlation between insulin resistance and serum homocysteine

Discussion:

Polycystic ovarian patients had been studied for age and anthropometric measurements (height, weight, waist circumference, hip circumference) compared to control group. It is obvious from Table 1, that there is no significant difference in age and anthropometric measurements between the two groups as all the subjects are age and BMI matched which the same was done by Salehpour *et al*. study [(11)]. This is important to rule out the effect of obesity on the studied parameters since recent studies showed that there were alterations in several metabolic pathways have been implicated in the pathophysiology of PCOS including abnormalities in steroid hormone regulation and insulin signaling pathway. Moreover, there was increasing focus on complications associated with metabolic disturbances among women with PCOS like obesity, Insulin resistance dyslipidemia and inflammation has been recognized as risk factors for developing diabetes mellitus and cardiovascular diseases in PCOS and also plasma homocysteine levels have been shown to correlate with BMI [(15,16,17)].

There were significant differences related to FSH, LH, and LH/FSH ratio between PCOS group and control group as shown in Table (2).It was obvious that LH and LH/FSH ratio were higher in PCOS group while FSH was lower in this group when it was compared to control. This study is similar to studies done by Cho *et al* and Saxena *et al* [(18, 19)] which revealed that PCOS patients had higher levels of LH and LH/FSH ratio while FSH was low.

There was no significant difference in testosterone levels between the two groups as shown in the same table, while Temel *et al* [(20)] founded an increase in free testosterone, since serum levels of free testosterone and not total testosterone were more frequently elevated in women with PCOS. Therefore serum free testosterone was the considered to be the most sensitive biochemical marker supporting the diagnosis of PCOS [(21)]. Measurement of total Testosterone in serum include a portion bound to sex hormone binding globulin (SHBG), because PCOS is often associated with decreased SHBG levels (because of obesity and insulin resistance), increased Testosterone clearance does not allow for an accurate reflection of increased androgen production [(22)]. In this study total testosterone was measured and not the free testosterone, this can explain the differences between our findings and Tamel *et al* finding.

There is significant difference regarding fasting blood sugar as shown in Table (3) this leads to the thought that PCOS patients were liable for hyperglycemia which is agreed with other study results [(20, 23, 24)], while other authors suggested that fasting glucose assessment alone not suffice for screening for pre-diabetes in women with PCOS because a single fasting blood level does not adequately identify those women in post-prandial glucose tolerance impairment test [(25, 26,27)].

Also Table (3) shows significant difference in serum insulin and HOMA-IR being higher in PCOS patients

compared to control and the results were similar to studies done by other authors[28,29] while Sills *et al* [30] could not find any connection between PCOS and insulin resistance.

As a consequence of sever insulin resistance, the risk of impaired glucose tolerance and type 2 diabetes mellitus in patients with PCOS significantly exceeds estimates of diabetes in normal population [28,30,31].

The same table demonstrates that serum homocysteine is significantly higher in PCOS patients than in the control group, this is in agreement with other studies [28,32], this may be due to obesity or chronic inflammation associated with PCOS[33] the current study doesn't agree with study done by Mancini *et al*.[34] who found no significant difference in homocysteine levels between PCOS and control groups. The lack of uniformity in the definition of PCOS and hyperhomocysteinemia can explain the differences between our findings and other studies.

There was no obvious correlation between IR and homocysteine within PCOS group as shown in the figure (1) possibly because they have different mechanism of action, similar study suggest that hyperhomocysteinemia in PCOS is independent of insulin resistance and is due to other factors [35] while Schachter *et al* [36] showed a significant correlation between homocysteine and IR levels regardless of body weight in women with PCOS. Evidences by Meigs *et al* [37] had shown that hyperinsulinemia and some phenotypes of insulin resistance syndrome could have several metabolic effects in general population, including hyperhomocysteinemia. It had been found that the relation between increased homocysteine levels and insulin resistance in particular groups of fertile women and examined the association of insulin resistance and hyperhomocysteinemia in preeclamptic pregnant women [38], Similar study reported that hyperhomocysteinemia is associated with hyperinsulinemia and partly accounted for increased risk of CVD associated with IR, direct association between hyperhomocysteinemia and IR concerning their similar pathogenic effects on vascular endothelial cells [37].

Conclusions:

Serum homocysteine levels and HOMA-IR are high in women with polycystic ovary syndrome compared to control, but there is no correlation between the two variables in PCOS women and each one may have a different mechanism of action.

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