

## Plasma Homocysteine and Insulin Resistance in Patients with Polycystic Ovary Syndrome

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### ABSTRACT:

#### BACKGROUND:

Polycystic Ovary Syndrome (PCOS) is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism and insulin resistance. Plasma homocysteine is a non-protein sulfur-containing  $\alpha$ -amino acid plays a role in cardiovascular morbidity and mortality due to its atherogenic and prothrombotic properties.

#### OBJECTIVE:

To assess the association between insulin resistance and homocysteine level in women suffering from polycystic ovary syndrome.

**Study design:** A case control study.

**Setting:** Department of Obstetrics and Gynecology at Al-Elwiya Maternity Teaching Hospital during the period from 1<sup>st</sup> of March 2017 to end of April 2018.

#### MATERIALS AND METHOD:

One hundred women suffering from polycystic ovary syndrome and same number of healthy women were included in the study.

After assessment according to questioner form, fasting blood sugar, serum insulin and plasma homocysteine were checked to find the association among them, the insulin resistance was calculated according to the following formula "IR= Fasting Glucose X Fasting Insulin/22.5= mmol/dl".

#### RESULTS:

Increase in body mass index is found to be accompanied with increase in mean fasting blood sugar (6.4±2.7 mmol/L), Insulin resistance (11.6±10.7 mmol/L) and homocysteine level (14.9±1.6  $\mu$ mol/L) when compared to control group whose mean values are (4.4±0.5 mmol/L, 3.6±1.5 mmol/L and 9±1.7  $\mu$ mol/L) respectively were significantly higher. A noticeable significant association between obesity and high fasting blood sugar (p0.04), high insulin resistance (p0.009) and homocysteine level (p0.001) in women suffering PCOS.

#### CONCLUSION:

There are significant differences found in homocysteine level and insulin resistance among women suffering from PCOS when compared to control group. A significant positive association between increased homocysteine level and insulin resistance with increased body mass index in women with PCOS.

**KEYWORDS:** PCOS, insulin resistance, homocysteine

### INTRODUCTION:

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by

multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism, which generate short and long term consequences on female health<sup>(1)</sup>.

The etiology of the syndrome remains obscure<sup>(2)</sup>, but there is a strong evidence that it can, to a large degree, be classified as a genetic disease. Excessive weight gain often precedes the appearance of PCOS features,<sup>(3)</sup> and healthy lifestyle that lowers body weight and abdominal fat will reduce testosterone levels and insulin

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resistance, and improves manifestations of the disease<sup>(4)</sup>.

Insulin resistance is defined as a reduced glucose-uptake response to a given amount of insulin. The mechanism of this decreased insulin sensitivity appears to be due to post-binding abnormality in insulin-receptor mediated transduction. Both thin and obese women with PCOS are found to be more insulin resistant than non-affected weight-matched controls. The hyperinsulinemic state present in most women with PCOS appears to play a central role in PCOS development and is considered to be the cause rather than the result of increased androgens<sup>(5)</sup>.

Homocysteine (Hcy) levels are positively correlated to the risk of cardiovascular disease and complications, by increasing oxidative stress in the vascular endothelium, activation of platelets, stimulation of vascular smooth muscle proliferation and may be one of the signals inducing apoptosis in vascular endothelial cells<sup>(6,7)</sup>. Investigations support that C-reactive protein, homocysteine and adiponectin are abnormal in women with polycystic ovary syndrome and considered to be the biomarkers of cardiovascular disorders<sup>(8)</sup>.

Elevated Insulin levels seem to influence Homocysteine via affecting the glomerular filtration or modifying enzymes activity involved in metabolism [i.e., Methyltetrahydrofolate Reductase and hepatic Cystathione  $\beta$ -Synthase<sup>(9)</sup>.

### **AIM OF THE STUDY:**

To assess the association between insulin resistance and homocysteine level in women suffering from Polycystic Ovary Syndrome.

### **PATIENTS AND METHODS:**

A case control study was carried out at AL-Elwiya Maternity Teaching Hospital in Baghdad. The study is conducted over a period from 1<sup>st</sup> of March 2017 to end of April 2018 and approved by the Obstetrics and Gynecology committee of Arabic Board for Medical Specialization. One hundred women suffering from polycystic ovary syndrome (already diagnosed according to their previous reports and further confirmation of diagnosis was done according to the Rotterdam criteria), they attended the gynecology clinic of the hospital seeking for medical advice because they were suffering from infertility or other manifestations of the disease. One hundred women with age matched healthy individuals were taken as the control group; they were arranged

according to their body mass index into three groups:

\*Group one (normal BMI from 18.5 to 24.9 kg/m<sup>2</sup>) which included: 20 women with PCOS compared to 25 healthy women.

\* Group two (overweight BMI from 25-29.9 kg/m<sup>2</sup>) which included: 39 women with PCOS compared to 48 healthy women.

\* Group three (obese BMI  $\geq$  30kg/m<sup>2</sup>) Participants with class I (BMI 30-34.9 kg/m<sup>2</sup>) and class II (BMI 35-39.9kg/m<sup>2</sup>) obesity were included in one group which included 41women with PCOS compared to 27 healthy women.

An informed consent explaining about the nature and the purpose of the study was obtained from the patients enrolled into the study before interference and blood samples collection.

### **Inclusion criteria**

1. Age from less than twenty years to above forty years.
2. Polycystic ovary syndrome according to Rotterdam criteria (5).

### **Exclusion criteria**

- 1- Smokers.
- 2- Previous ovarian surgery.
- 3- Recent hormonal therapy.
- 4- Evident medical diseases according to their detailed history.

The study protocol included a questionnaire form designed to include details about age, menstrual cycle pattern, fertility status, hair growth and distribution, weight, height, acne, endocrine abnormalities (hormonal profile), medical history, surgical history and ultrasound details. General examination was done for the participants including vital signs, also anthropometrics measurements (weight, height) for Body Mass Index (BMI) calculation (3).

$$\text{BMI} = \frac{\text{weight in kg}}{\text{square of height in meters}}$$

Ultrasound was done for the participants looking for features of polycystic ovary syndrome and blood sample was taken from them to measure plasma homocysteine, serum insulin and fasting blood sugar levels.

### **Blood sample collection and preparation**

Five mls of venous blood was taken from the participants after fasting 8 hours, at any day of menstrual cycle since homocysteine level, insulin hormone and blood sugar level remain stable throughout the menstrual cycle.

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The blood sample was centrifuged at 3000 RPM for 15 minutes and the serum obtained in properly stored at - 40 ° C till analysis. Homocysteine was measured using enzyme-linked-immunosorbent sensitive assay kit (HOMOCYSTEINE ELISA)

The insulin resistance was calculated as:

IR= Fasting Glucose X Fasting Insulin/22.5= mmol/dl<sup>(10)</sup>.

Normal fasting blood sugar (60-90mg/dl). Normal fasting serum insulin (2-25mmol/l)<sup>(11)</sup>. Normal plasma homocystiene level (5-11Mmol/ml).<sup>(12)</sup>

### Statistical analysis

The data obtained were analyzed by using Microsoft excel program and Statistical Package for Social Sciences (SPSS) version 23.

Outcomes of analysis were arranged in scales variables (means & standard deviation) and in categorical variables. Chi square test was used for comparison between categorical data (Fishers exact test applied when expected variable was less than 20% of total). Independent sample t-test was used to compare between two means. The level of significance (p value) was set as  $\leq 0.05$ .

### RESULTS:

This study included 100 PCOS women and 100 healthy women served as controls. The mean age of PCOS women was 26.9±5.7 years, while of controls was 28.9±6.1 with a significant difference between them (p=0.01) as shown in table 1.

**Table 1: Distribution of age according to study groups.**

Variable	PCOS women		Controls		P
	No.	%	No.	%	
<b>Age</b>					
<20 years	16	16.0	15	15.0	0.3*Not significant
20-29 years	54	54.0	45	45.0	
30-39 years	24	24.0	36	36.0	
≥40	6	6.0	4	4.0	
Mean ± SD (years)	26.9±5.7		28.9±6.1		0.01**

\*Fishers exact test, \*\*Independent sample t-test.

No significant differences were observed between women with PCOS and healthy controls regarding their BMI groups (p=0.1), however, mean BMI of

women with PCOS was significantly higher than mean BMI of healthy controls (p=0.01) as shown in table 2.

**Table 2: Distribution of body mass index according to study groups.**

Variable	PCOS women		Controls		P
	No.	%	No.	%	
<b>Body Mass Index</b>					
Normal	20	20.0	25	25.0	0.1*Not significant
Overweight	39	39.0	48	48.0	
Obese	41	41.0	27	27.0	
Mean±SD (years)	29.1±4.9		27.5±4.9		0.01** Significant

\*Fishers exact test, \*\*Independent sample t-test.

The mean FBS of women with PCOS was significantly higher than mean FBS of controls (p<0.001) and mean fasting insulin level of women with PCOS was significantly higher than mean

fasting insulin level of controls (p<0.001). Mean insulin resistance level of women with PCOS was significantly higher than mean insulin resistance level of controls (p<0.001).

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**Table 3: Distribution of fasting glucose & insulin and insulin resistance according to study groups.**

Variable	PCOS women		Controls		P
	No.	%	No.	%	
Fasting blood sugar					<0.001* Significant
Normal	56	56.0	99	99.0	
High	44	44.0	1	1.0	
Mean±SD (mmol/L)	5.8±1.9		4.4±0.5		<0.001**
Fasting insulin					<0.001* Significant
Normal	46	46.0	87	87.0	
High	54	54.0	13	13.0	
Mean±SD (IU/L)	29.8±20.6		18.1±5.8		<0.001**
IR					<0.001** Significant
Mean±SD (mmol/L)	8.6±8.2	3.6±1.5			

\* Chi-square test, \*\* Independent sample t-test.

The mean homocysteine level in women with PCOS is shown to be higher than mean homocysteine of controls ( $p < 0.001$ ).

**Table 4: Distribution of homocysteine level according to study groups.**

Variable	PCOS women		Controls		P
	No.	%	No.	%	
Homocysteine level					<0.001* Significant
Normal	39	39.0	100	100.0	
High	61	61.0	0	-	
Mean±SD (µmol/L)	14.2±1.7		9±1.7		<0.001**

\* Chi-square test, \*\* Independent sample t-test.

There was a significant positive association resistance among women suffering from PCOS between high homocysteine level and Insulin ( $p < 0.001$ ).

**Table 5: Association between Homocysteine level and Insulin resistance.**

variable	PCOS Women	Control	P-value
	Mean±SD	Mean±SD	
Insulin resistance	8.6±8.2	3.6±1.5	< 0.001
Homocysteine	14.2±1.7	9±1.7	< 0.001

There was significant association between higher fasting blood sugar level and obese women with PCOS ( $p = 0.04$ ). There was highly significant association between higher mean level of homocysteine and obesity in women with PCOS ( $p < 0.001$ ). A significant association was observed between higher IR level and obesity in women with PCOS.

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**Table 6: Association between BMI and different variable in women with PCOS.**

BMI	Fasting sugar	Homocysteine	Insulin resistance
	Mean±SD	Mean±SD	Mean±SD
Normal	5.6±0.4	12.5±0.9	8.1±5
Overweight	5.4±0.9	14.3±1.5	5.7±4.3
Obese	6.4±2.7	14.9±1.6	11.6±10.7
P value *	0.04 <sup>S</sup>	<0.001 <sup>HS</sup>	0.009 <sup>S</sup>

\* One-way ANOVA analysis, S=Significant

### DISCUSSION:

Mean age of the PCOS women was 26.9±5.7 years and 28.9.1±6.1 for the controls and mean BMI was (29.1±4.9) for the PCOS women compared with (27.5±4.9) for the controls with a significant statistical difference (p 0.01) seen between the two means, and it seems that overweight women are more likely affected by PCOS which corresponds with a study of Schachter et al<sup>(10)</sup>, Who also found a noticeable difference between plasma homocysteine and insulin resistance between both groups and also goes with the study of sachan<sup>(12)</sup> Who enrolled 90 women 45 of them were diagnosed with PCOS and 45 women as control and found the mean of homocysteine and insulin resistance value in PCOS women were significantly higher than those in control group. But in a recent study done by Upadhye J. in 2017 revealed that the mean age group was in between 20-24 years and that more than half of the respondents are within normal weight and only 19.5% of them were overweight<sup>(13)</sup>, this probably dues to difference in sample size collection.

The study reports a significant association between insulin resistance and overweight in women with PCOS, which is nearly similar to results of Diamanti-Kandarakis E et al 2009, and Pasquali R et al in 2006, when they stressed that lifestyle-related metabolic diseases, mainly overweight-obesity and insulin resistance was verified to exacerbate PCOS<sup>(14,15)</sup>

Menstrual irregularities and unpleasant menstrual symptoms have also been revealed to be an area of great concern and distress. Barnard et al., 2007, discovered that menstrual symptoms were the second largest area of concern in PCOS women<sup>(16)</sup>. The present study resulted that menstrual irregularities were more common in overweight-obese POCs women.

According to the data obtained, there was statistically significant difference between mean

fasting blood sugar in PCOS women 5.8±1.9, and their controls (4.4±0.5); the mean fasting insulin in PCOS was 29.8±20.6 higher than control 18.1±5.8. These findings seem to be in agreement with the findings of Badawy 2007<sup>(17)</sup>, while it differs from a study done by Schachter in 2003<sup>(10)</sup>, who concluded that insulin resistance and hyperinsulinemia seen in women with PCOS is accompanied with increase plasma homocysteine regardless body weight and explained its effect on infertility and on the long term cardiovascular and metabolic disorder seen in women with untreated disease.

Regarding homocysteine level there was higher mean homocysteine level 14.2±1.7 in women with PCOS compared to control group mean is 9±1.7 which goes with Salehpour 2011<sup>(8)</sup> who enrolled in his study 85 PCOS women and 83 controls matched by body mass index (BMI), homocysteine levels were assessed. The mean level of homocysteine was 16.25 ± 11.94 µmol/L in women with PCOS and 11.58 ± 3.82 µmol/L in controls (p=0.002) also Maleedhue 2014<sup>(18)</sup> stated that homocysteine levels are significantly higher in women with PCOS than control group. While the figures differ from study of Killiks 2004<sup>(19)</sup> who said, no correlation between the presence of insulin resistance, homocysteine in women with PCOS. These different correlations reported by different researches may be related to the differences between insulin resistance, homocysteine definition, duration of the study, selection bias and being single sample of blood studied. According to our result as shown in (table 6), there is a noticeable significant association between increase body mass index, high fasting blood sugar (p0.04), high insulin resistance (p0.009) and homocysteine level (p0.001) in women suffering from PCOS. This coincides with study of Sedigheh E.in 2017<sup>(20)</sup>.

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who enrolled 60 women with PCOS and 20 healthy women and found that women with PCOS show the tendency toward hyperhomocysteinemia, hyperinsulinemia and above normal BMI. While Yarali showed that increased homocysteine in those women are related to insulin resistance and not body weight<sup>(21)</sup>.

### CONCLUSION:

The current study concluded:

1. A significant difference in homocysteine level and insulin resistance among women suffering from PCOS when compared to control group.
2. A significant positive association between increase homocystiene level and insulin resistance with increased body mass index in women with PCOS.

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