

Studying Levels of Homocysteine in Diagnosed Cases of Polycystic Ovary Syndrome

Rojeen Rasheed Suleiman, Dhia Mustafa Sulaiman

Department of Medical Laboratory Technology, Shekhan Technical College of Health, Duhok Polytechnic University, Duhok, Iraq

Abstract

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by an ovulatory infertility, menstrual dysfunction, and hirsutism. The pathophysiology behind PCOS is complex. Comorbidities associated with PCOS are hypertension, diabetes, dyslipidemia, and cardiovascular events. **Objective:** The aim of this study was to estimate the level of homocysteine in women with PCOS. **Materials and Methods:** In the current case-control study, 50 female patients diagnosed with PCOS and 40 apparently healthy controls were recruited. The diagnosis of PCOS was established according to Rotterdam criteria. The serum homocysteine level was measured using Cobas 6000 and compared between two study groups. **Results:** Results found that high percentage of patients in the case group had abnormal level of homocysteine (74.0%) compared to control with normal level of homocysteine. Mean homocysteine level was significantly higher in patients with PCOS (17 $\mu\text{mol/L}$) compared to its level in control patients (9 $\mu\text{mol/L}$), $P < 0.0001$. Results also revealed that the mean levels of homocysteine between the body mass index categories and marital status showed no significant difference in cases and controls, $P > 0.05$. **Conclusions:** The present investigation suggests that higher level of serum homocysteine could be used as a biomarker for PCOS.

Keywords: Biomarkers, homocysteine, patients, polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by an ovulatory infertility, menstrual dysfunction, and hirsutism.^[1] The pathophysiology of PCOS is unclear. The comorbidities associated with PCOS are hypertension, diabetes, dyslipidemia, and cardiovascular events.^[2] It is associated with various clinical features including amenorrhea, oligomenorrhea, hirsutism, obesity, infertility, anovulation, and acne.^[3] The prevalence of disease is 5%–10% depends on ethnic origin, race, environmental factor, and phenotype.^[4]

PCOS is not a gynecological disease alone; it is associated with obesity, insulin insensitivity, and metabolic syndrome leading to future health problems such as cardiovascular events and diabetes mellitus.^[5] The criteria for diagnosis of PCOS vary and the most commonly used criteria is Rotterdam criteria by the presence of at least two out of three criteria: oligoanovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries (≥ 12 follicles measuring 2–9 mm in diameter, or ovarian volume 0.10 ml in at least one ovary).^[6]

Homocysteine is sulfur-containing nonproteinogenic amino acid formed from breakdown of methionine either transulfate to cystathionine or remethylate to methionine. There is some evidence that the level of homocysteine is abnormal in patients with PCOS.^[7] Homocysteine metabolizes through two pathway either methylation to methionine, which requires folate and Vitamin B12 or transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate.^[8] Homocysteine is essential amino acid that requires for growth of cell and tissue. The only source of homocysteine in human is methionine, which is present in dietary proteins of animal origin.^[9]

It is claimed that some factors are related with homocysteine level such as age, sex, smoking, physical activity, chronic inflammation, nutrition, and insulin.^[10] Homocysteine is contributes to early miscarriage due to impair implantation

Address for correspondence: Dr. Dhia Mustafa Sulaiman, Department of Medical Laboratory Technology, Shekhan Technical College of Health, Duhok Polytechnic University, Duhok, Iraq. E-mail: dhiamizori@yahoo.com

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through interfering with the endometrial blood flow and its vascular integrity.^[11]

The studies accent the possible role of homocysteine in the occurrence of PCOS in women populations. Homocysteine has portrayed as an independent risk factor for disease of heart and vascular tree.^[12] Therefore, the raised homocysteine level is possible another element of PCOS add to expand extensively cardiovascular disorder.^[13] There is an evident gap on identification of homocysteine levels in patients diagnosed with PCOS in this region.

The homocysteine levels in patients with PCOS in a sample of Iraqi patients were assessed and evaluated in the current study. In addition, the serum homocysteine level and its association with body mass index (BMI) and marital status was evaluated in this study.

MATERIALS AND METHODS

Study design and sampling

A case-control study was conducted among 50 female patients diagnosed with PCOS and their homocysteine levels were compared with 40 healthy controls as control. In the present study, the cases were selected from the patients consecutively visited outpatient clinics of Azadi Teaching Hospital - Duhok in 2018. The patients' relatives and their companions were considered as healthy controls and recruited in control group following performing detail physical and clinical examinations. Samples were collected from Duhok maternity hospital between May 2017 and April 2018.

Inclusion and exclusion criteria

All women in the reproductive age 15–44 years were eligible to participate in this study. The patients with diabetes mellitus, hypertension, coronary heart disease, endocrine disorders, alcoholic consumption, renal diseases, and liver disease were excluded from the study. In addition, the patients were smoking, on pregnancy, and those taking multivitamins were not included in the study.

Diagnostic and measurement criteria

The diagnosis of PCOS was established in line with the Rotterdam criteria by an experienced gynecologist or obstetrician by the presence of at least two out of three criteria: oligoanovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries (≥ 12 follicles measuring 2–9 mm in diameter, or ovarian volume 0.10 ml in at least one ovary). Then, the level of homocysteine is measured by Cobas 6000 analyzer series on the base of immunoassay. Hyperhomocysteinemia was classified as follows: mild (15–30 $\mu\text{mol/L}$), intermediate (30–100 $\mu\text{mol/L}$), and severe (>100 $\mu\text{mol/L}$).^[14]

Statistical methods

The descriptive purposes of the present study were determine through the frequency distribution whether mean and standard deviation or frequency and percentage. The difference of homocysteine levels between cases and controls was examined

through the Mann–Whitney U-test with taking into account the significant level at $P < 0.05$. The statistical calculations were performed using Statistical Package for the Social Sciences version 24 (SPSS, IBM Company, Chicago, USA).

Ethical considerations

The ethical approval of the present study was obtained from the Kurdistan Board of Medical Specialties (KBMS). The guarantee was given to the patients for the confidentiality of their personal information.

RESULTS

The cases and controls were comparable (similar) in age, 26.88 years versus 27.58 years, respectively, ($P = 0.620$) and marital status ($P = 0.924$). However, the BMI of cases was significantly higher (27.17) compared to BMI of controls (24.24), $P = 0.002$. In addition, a greater percentage of patients in case group had abnormal level of homocysteine (74.0%) compared to the higher percentage of patients in control with normal level of homocysteine (87.5%), ($P < 0.0001$) [Table 1].

The study showed that homocysteine concentration level in cases was significantly higher than it is patients in control group (17.00) versus (9.00), $P < 0.0001$ [Table 2].

The mean levels of homocysteine between the BMI categories and marital status did not show the significant difference in cases and controls, $P > 0.05$ [Table 3].

Table 1: Difference of general characteristics between cases and controls

General characteristic	Study groups		P (two-sided)
	Mean±SD, frequency (%)		
	Cases (n=50)	Controls (n=40)	
Age (year)	26.88±6.76	27.58±6.45	0.620*
BMI	27.17±5.24	24.24±3.39	0.002*
Marital status			
Married	22 (44.0)	18 (45.0)	0.924**
Single	28 (56.0)	22 (55.0)	
Homocysteine categories			
Normal	13 (26.0)	35 (87.5)	<0.0001**
Abnormal	37 (74.0)	5 (12.5)	

*Independent *t*-test, **Chi-squared test were performed for statistical analysis. SD: Standard deviation, BMI: Body mass index

Table 2: Difference of homocysteine levels between cases and controls

General characteristic	Study groups, median $\pm$ IQR		*P (two-sided)
	Cases (n=50)	Controls (n=40)	
Homocysteine level (micromole/L)	17.00 $\pm$ 4.00	9.00 $\pm$ 2.15	<0.0001

*Mann–Whitney U-test was performed for statistical analysis.

IQR: Interquartile range

Table 3: Comparison of homocysteine between body mass index categories and marital status in cases and controls

Patient type	Mean $\pm$ SD	
	Cases (n=50)	Controls (n=40)
BMI		
Underweight	15.75	8.25
Normal weight	16.00 $\pm$ 8.65	9.0 $\pm$ 2.80
Overweight/obese	17.00 $\pm$ 3.95	9.00 $\pm$ 2.00
P*	0.716	0.876
Marital status		
Single	17.00 $\pm$ 3.88	10.38 $\pm$ 16.50
Married	8.25 $\pm$ 2.28	9.00 $\pm$ 1.53
P**	0.499	0.815

*Kruskal–Wallis test, **Mann–Whitney U-test were performed for statistical analyses. BMI: Body mass index, SD: Standard deviation

DISCUSSION

Our data demonstrated that the females with PCOS have a significantly higher rate of hyperhomocysteinemia (17 $\pm$ 4.00 vs. 9 $\pm$ 2.15, respectively, $P < 0.0001$) and higher mean serum homocysteine level compared to healthy females (17.00 vs. 9.00, respectively). This may be due to insulin resistance by its effect on cystathionine β -synthetase. The prevalence of hyperhomocysteinemia in case with PCOS was 74% compared to control 12.5%, which is a statistically significant percentage.

These results agree with several previous studies conducted worldwide which revealed higher homocysteine concentrations in PCOS than control. The case-control study of Al-Gareeb *et al.* showed that serum homocysteine levels were significantly high in PCOS.^[15] However, these results disagree with another study that did not determine significant differences in homocysteine levels between women with PCOS and control group.^[16]

Maleedhu *et al.* in their cross-sectional study on 142 cases and 65 controls reported a significant increase of mean serum homocysteine level in normal and obese cases compared with respective controls. The significant incremental increase of serum homocysteine was observed from normal controls, obese controls, normal cases, and obese cases.^[17] Yaralı, Hakan *et al.* on their study reveal that mean serum homocysteine concentration was significantly higher in the polycystic ovary syndrome group (11.2 $\pm$ 4.1 μ mol/L vs. 9.0 $\pm$ 2.4 μ mol/L, $P = 0.01$).^[18]

Boulman *et al.* in their study show that there was no difference between the PCOS and control groups in glucose, lipoproteins, thyroid-stimulating hormone, homocysteine, or in WBC concentrations.^[19] Orio *et al.* on their study reveal that no difference in mean homocysteine levels was observed between PCOS women in comparison to the control group.^[20]

Obesity plays a role in the development of PCOS, which has negative an impact on PCOS development.^[21] The present

study demonstrated that the BMI of patients with PCOS was significantly higher than control $P = 0.002$, which is concurrent with earlier studies, which found that increased BMI is a characteristic finding in women with PCOS.^[22] Esmaeilzadeh *et al.* on their study reveal significant relation between BMI and PCOS.^[23] This could be a consequence of higher level of androgen.

In the present study, serum homocysteine between cases with normal BMI and abnormal BMI (obese) was not significant (16.00 $\pm$ 8.65, 17.00 $\pm$ 3.95). The prevalence of hyperhomocysteinemia in relation to BMI is 75% in cases and 25% in controls. Guzelmeric *et al.* on their study revealed an increased BMI is associated with an increased level of homocysteine.^[18] In multiple study, age and BMI were not predictors for hyperhomocysteinemia. Kilic-Okman *et al.* on their cross-sectional study revealed no relation between an increase mean homocysteine level and BMI.^[24]

In our data, there was a decreasing level of homocysteine among married cases compared to single cases of PCOS (17.00 $\pm$ 3.88, 10.38 $\pm$ 16.50).

Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular diseases, cerebrovascular events, and recurrent venous thromboembolism. It can occur due to genetic defects in the enzymes involved in homocysteine pathways such as methylenetetrahydrofolate reductase, to deficiencies in vitamin cofactors, or additional factors, which include certain chronic medical conditions and drugs, such as fibrates and nicotinic acid.^[25]

PCOS is associated with insulin resistance which results in hyperhomocysteinemia due to its effect in enzyme cystathionine β -synthetase.^[26] Insulin resistant was not assayed in this study; however, previous studies showed a correlation between homocysteine level and insulin resistant (IR) in PCOS. Sachan *et al.* on their cross-sectional study on 50 cases of PCOS and 40 controls found that among the PCOS patients (32/50), 64% were insulin resistant and (18/50), 36% were non-IR PCOS group which was stratified by Homeostatic model assessment-IR.^[27] Hemati *et al.* on their prospective study on 50 cases of PCOS show the IR group had significantly higher homocysteine ($P = 0.02$), fasting insulin, and glucose levels ($P < 0.001$) rather than NIR group.^[28]

CONCLUSION

The present investigation suggests that serum level homocysteine can be used as a biomarker for PCOS diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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