Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2024; 41(3): 646-652
doi: 10.52142/omujecm.41.3.33

Serum magnesium and C-peptide levels in Iraqi women with polycystic ovary syndrome

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Received: 02.06.2024 • **Accepted/Published Online:** 31.07.2024 • **Final Version:** 30.09.2024

Abstract

Magnesium (Mg) is the second most frequent intracellular cation in humans with critical role in insulin metabolism and glucoregulation. It is an antioxidant and acts as a cofactor for several enzymes. Evaluate serum Mg levels Iraqi women with PCOS and assess its relationship with C-peptide and BMI. This is a case control study that included 80 women with PCOS and 80 healthy volunteers (as control group) in the age range between 18-45 years old. C-peptide was measured using ELISA technique, while Mg was measured using a spectrophotometric technique. Serum C-peptide levels were significantly higher in PCOS patients compared to the control group. In PCOS patients, serum magnesium concentration was significantly lower compared to the control group. This experiment found no correlation between Mg, C-peptide, or BMI. The ROC analysis area under the curves for Mg and C-peptide were 0.803 and 0.875, respectively. These figures demonstrate analysis method reliability. PCOS patients had lower blood magnesium and higher C-peptide levels. No statistically significant relationship was found between these factors. Due to the low magnesium levels in PCOS women's serum. The correlation between lower serum magnesium and higher C-peptide levels in PCOS patients was not significant. Lower serum magnesium levels in women with PCOS need further study and treatment to determine its role and association with PCOS.

Keywords: magnesium, C-peptide, polycystic ovary syndrome, ELISA

1. Introduction

Polycystic ovarian syndrome (PCOS) is a common and diverse illness characterized by symptoms that vary depending on age and require personalized treatment approaches. The syndrome is defined by excessive levels of male hormones (hyperandrogenism) together with irregular or infrequent menstrual cycles (chronic oligo-anovulation), small cysts on the ovaries (micropolycystic morphology), and impaired ovarian function (1). Approximately 75% of women with polycystic ovary syndrome are affected by anovulatory infertility. Additionally, individuals who undergo in vitro fertilization (IVF) have a higher likelihood of developing ovarian hyperstimulation syndrome. Moreover, it is important to highlight that these women are also at a significantly higher risk of developing gestational diabetes and experiencing miscarriage (2). Polycystic ovary syndrome (PCOS), despite being rather common, is frequently undetected and often requires numerous consultations with different doctors or visits. The process of identifying PCOS usually takes more than a year. The process is quite vexing for the patient. Delaying the identification of comorbidities can hinder the adoption of lifestyle treatments, which are essential for improving PCOS symptoms and improved quality of life (3, 4). Personality disorder 2 (PCOS) is associated with various negative health issues, including cardiovascular risk, depression, obstructive sleep apnea (OSA), endometrial cancer, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, infertility, and metabolic syndrome. There are different screening recommendations for each of these diseases. However, doctors should be vigilant and conduct further inquiry in patients with polycystic ovary syndrome (5, 6) if any symptoms are present. Functional ovarian hyperandrogenism (FOH) is the primary cause of the majority of PCOS patients. Functional ovarian hyperandrogenism, observed in about two-thirds of instances of polycystic ovary syndrome (PCOS), is characterized by an excessive response of 17-hydroxyprogesterone (17-OHP) to gonadotropin stimulation and abnormal control of androgen production. In cases with residual polycystic ovary syndrome (PCOS) with atypical functional ovarian hyperandrogenism (FOH), there is no excessive response of 17-hydroxyprogesterone (17-OHP). However, an increase in testosterone levels can indicate the presence of atypical FOH if the production of adrenal androgens is suppressed. Approximately 3% of individuals diagnosed with PCOS also exhibit isolated functional adrenal

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hyperandrogenism (7). These individuals do not show any signs of abnormal steroid production; most of them are overweight, which doctors believe may be the reason for their unusual PCOS symptoms. Currently, conducting targeted tests for the FOH subgroup has minimal clinical usefulness (8, 9). Insulin acts as the primary hormone that controls the balance of glucose in the body and the production of lipids. Insulin acts as a hormone that stimulates cellular growth, in addition to its effects on the metabolism of carbohydrates, lipids, and proteins (10). Insulin's effects are carried out by insulin receptors, which are present in multiple tissues along the hypothalamic-pituitary-ovarian axis. Insulin increases the action of trophic hormones that are associated with steroidogenesis in steroidogenic tissues such as the ovary and adrenal cortex (11, 12). Hyperinsulinemia is the main cause of increased androgen synthesis because insulin indirectly enhances GnRH and mimics the effects of LH. Insulin decreases the amounts of sex hormone binding globulin (SHBG), a crucial protein in the bloodstream that regulates testosterone levels. Therefore, lower levels of SHBG would lead to higher amounts of unattached androgens, which are responsible for the appearance of clinical symptoms related to PCOS, such as excessive hair growth, hair loss, and acne (13, 14). There is a lot of documentation available that shows a strong connection between magnesium (Mg) and insulin resistance (IR) in people who have been diagnosed with metabolic syndrome and diabetic mellitus (DM) (15, 16). To be more precise, there is a correlation between a lack of magnesium and insulin resistance (IR). The aim of this study was to assess the serum levels of magnesium and C-peptide in patients with polycystic ovarian syndrome (PCOS), compared to control subjects who did not take magnesium supplements during the study period. Furthermore, explore the correlation between magnesium levels, C-peptide, and body mass index in individuals diagnosed with PCOS.

2. Materials and methods

This is a case-control study that was conducted in Department of Chemistry and Biochemistry at the College of Medicine/Al-Nahrain University and the Gynecological out-patient clinic at AlImmamain Alkadhmain Medical city during the period from 1/12/2021 to 1/9/2022. The participants included 80 PCOS women and 80 controls aged 18–45 years. Women with inflammatory conditions, congenital adrenal hyperplasia, hyperprolactinemia, Cushing's syndrome, and women taking Mg supplementation or other medication, were excluded from the study. Each participant was subjected to a physical examination. All individuals had their height and weight measured being the most widely accepted, the Rotterdam criterion is currently used to diagnose policy. The body mass index (BMI) is measured in kilograms per square meter. The diagnosis of polycystic ovarian syndrome (PCOS) was made based on the Rotterdam criteria. It requires the selection of two of the three following criteria: 1. oligo or anovulation, 2. clinical and/or biochemical signs of hyperandrogenism, and 3. polycystic ovaries (either 12 or more follicles measuring 2–9 mm in diameter, or an ovarian volume of >10 cm3). Approximately 5mL of venous blood was withdrawn from all participants and converted into a serum separated tube (Gel Tube) for magnesium and C-peptide measurement. Serum sample preparation was done by letting the whole blood sit for 20-30 minutes at room temperature (25°C) then the sample was centrifuged at 2000-3000 rpm for 20 minutes. The separated serum was stored in deep freeze (-20ºC) for subsequent measurement of serum magnesium (by using Serum Magnesium kit/ Linear /Germany) and C-peptide (by using Serum Human C-Peptide kit/ BioSource/USA).

2.1. Statistical analysis

SPSS for Windows, version 26, was employed to evaluate the findings (SPSS Inc. Chicago, Illinois, United States). The normality distribution of all variables was analyzed. The findings were tabulated as mean and standard deviation (SD). Following the ANOVA test, the Post Hoc test was employed. Pearson correlation was implemented to investigate the relationship degree between various parameters. $P<0.05$ was designated as the threshold for significance.

3. Results

3.1. Comparison of the study parameters between PCOS patients and controls

This study showed no significant difference in BMI between PCOS (26.10±4.12) and control (26.08±3.43) groups. The mean ages of the control and PCOS groups were (29.61±7.09) and (29.00±6.49) years, respectively. The two-tailed independent samples t-test result was not significant, $t(158) = -0.51$, $p=0.609$, indicating that the mean age was not significantly different between the PCOS and control groups. The level of C-Peptide for PCOS patients (3.53±1.04 ng/ml) was significantly higher than for control group (2.20±0.86 ng/ml), $t(152.69) = 8.79$, $p<0.001$. A two-tailed independent samples t-test was conducted to examine whether the mean of magnesium was significantly different between the PCOS and control groups. The serum level of magnesium for PCOS (1.06±0.23 mmol/L) was significantly lower than for control group (1.48±0.75 mmol/L), $p<0.001$. Instead of using Student's t-test, which is less reliable when dealing with different sample sizes and variances between two samples (Ruxton, 2006) (17), Welch's t-test was used. With an α value of 0.05 and a t-test result for two-tailed independent samples [$t(93.06) = -4.81$, $p<0.001$], the statistical analysis indicates that the null hypothesis can be rejected due to the high degree of significance. The finding demonstrates a substantial difference in the average magnesium levels between the PCOS and control groups. The results are presented in Table 1, while the bar charts illustrating the average values for each parameter may be seen in Fig. 1-4.

Table 1. Comparison of the study parameters between PCOS patients

and controls

Variable	*PCOS*	*Control*	two-tailed independent samples *t*-test		
	M±*SD*	*M*±*SD*	*t*	*p*	*d*
Age (Years)	29.06±6.47	29.61±7.09	-0.51	.609	0.08
BMI	26.15±4.11	26.08±3.43	0.11	.914	0.02
C-Peptide (ng/ml)	3.53±1.04	2.20±0.86	8.79	**<.001**	1.39
Magnesium (mmol/L)	1.06±0.23	1.48±0.75	-4.81	**<.001**	0.76

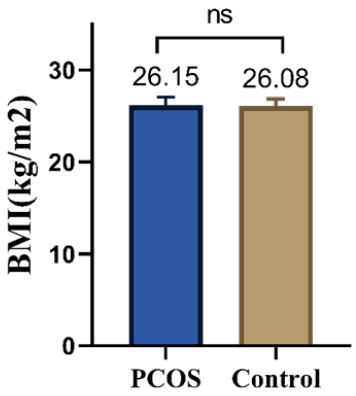

Fig. 1. The mean of BMI (ns: non-significant difference)

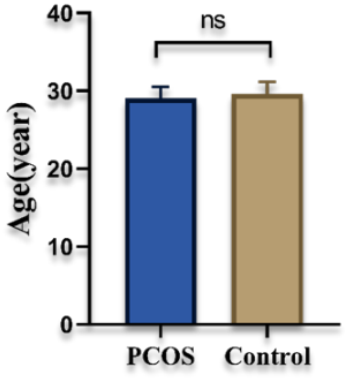

Fig. 2. The mean of Age (ns: non-significant difference)

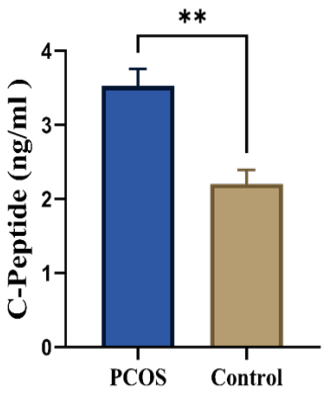

Fig. 3. The mean of C-peptide (**: significant difference)

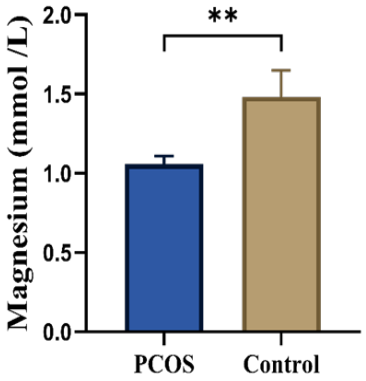

Fig. 4. The mean of Mg (**: significant difference)

3.2. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in PCOS Group

The results pertaining to the relationship between magnesium and C-peptide levels in women with polycystic ovarian syndrome indicate that the connection is feeble and does not possess statistical importance. The Spearman correlation coefficient (r) for this group was -0.03, with a 95% confidence range ranging from -0.25 to 0.19. The value of (p) is 1.000. Likewise, the relationship between magnesium and body mass index (BMI), as well as magnesium and age, showed no statistical significance with correlation coefficients of .13 with similar confidence intervals and p values equal to 1.000. It was also noted that there was no statistical correlation between C-peptide and body mass index, with a correlation coefficient of -.06, a confidence interval of [-.27, .17], and a p value of 1.000. While a strong and statistically significant negative correlation was recorded between C-peptide and age, with a correlation coefficient (r) equal to -.79, a confidence interval ranging between [-.86, -.70] and a value (p) of less than .001. Finally, a weak correlation was found between body mass index and age, with a correlation coefficient equal to .16, a confidence interval between [-.06, .37] and a (p) value equal to 1.000, which indicates that there is no statistical significance for this correlation (Table 2, Fig. 5).

Table 2. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in PCOS Group

Combination	*r*	95.00% CI	*p*
Magnesium-C-Peptide	-.03	[-.25, .19]	1.000
Magnesium-BMI	.13	[-.09, .34]	1.000
Magnesium-Age	.13	[-.10, .34]	1.000
C-Peptide-BMI	-.06	[-.27, .17]	1.000
C-Peptide-Age	-.79	[-.86, -.70]	< .001
BMI-Age	.16	[-.06, .37]	1.000

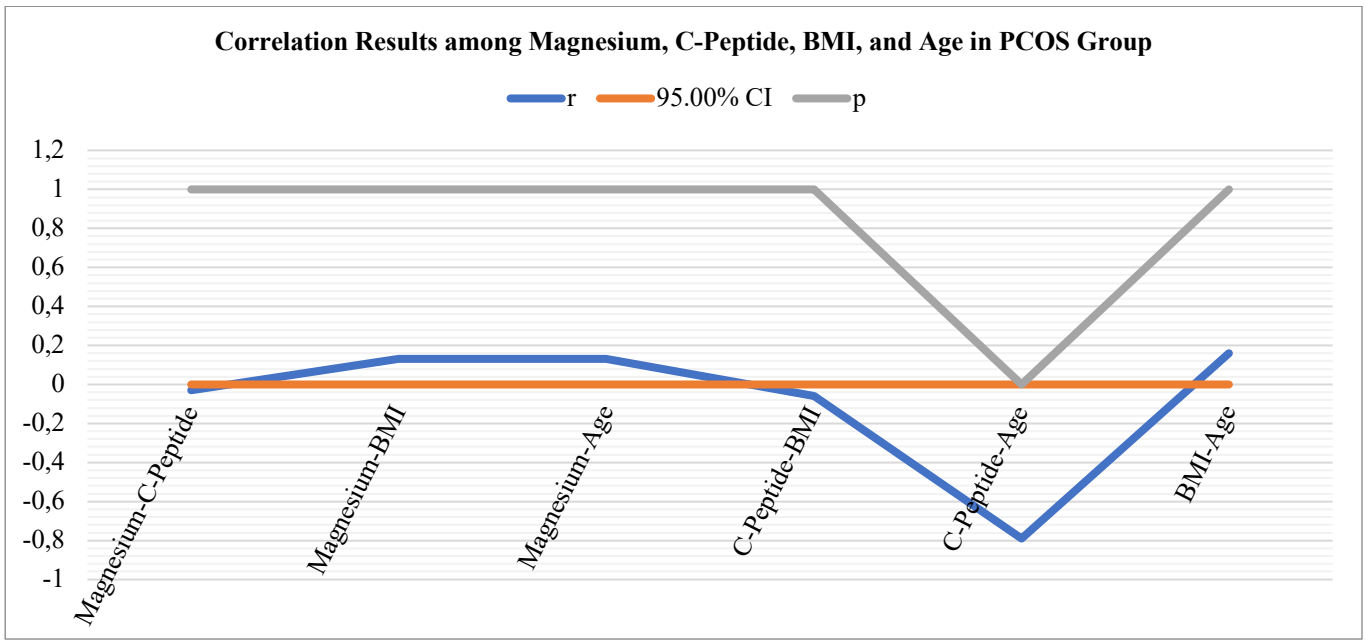

Fig. 5. Spearman Correlation Results in PSOC

3.3. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in control Group

In the statistical correlation study of the control group, the results show a very weak correlation between magnesium and C-peptide levels (r=.03) with a 95% confidence interval of [-.19, .25] and a p-value of 1.000, indicating no evidence A statistic that indicates an association. The relationship between magnesium and body mass index (BMI) was also weakly negative (r=-.06) according to a 95% confidence interval of [-.28, .16] with p=1.000. While there was a weak correlation between magnesium and age (r=.15) with a confidence interval ranging between [-.08, .36] with p=1.000. The results indicated a negative correlation between C-peptide and BMI (r=-.14) with a confidence interval [-.35, .09] and p=1.000. However, the negative correlation between C-peptide and age was more pronounced (r=-.52) with a confidence interval ranging between [-.66, -.34] and a p-value less than .001, indicating a significant and strong statistical correlation between them. Finally, the table showed no statistically significant correlation between BMI and age (r=.10) with a confidence interval [-.12, .32] and p=1.000 (Table 3, Fig. 6).

Table 3. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in control Group

Combination	r	95.00% CI	p
Magnesium-C-Peptide	.03	[-.19, .25]	1.000
Magnesium-BMI	-.06	[-.28, .16]	1.000
Magnesium-Age	.15	[-.08, .36]	1.000
C-Peptide-BMI	-.14	[-.35, .09]	1.000
C-Peptide-Age	-.52	[-.66, -.34]	< .001
BMI-Age	.10	[-.12, .32]	1.000

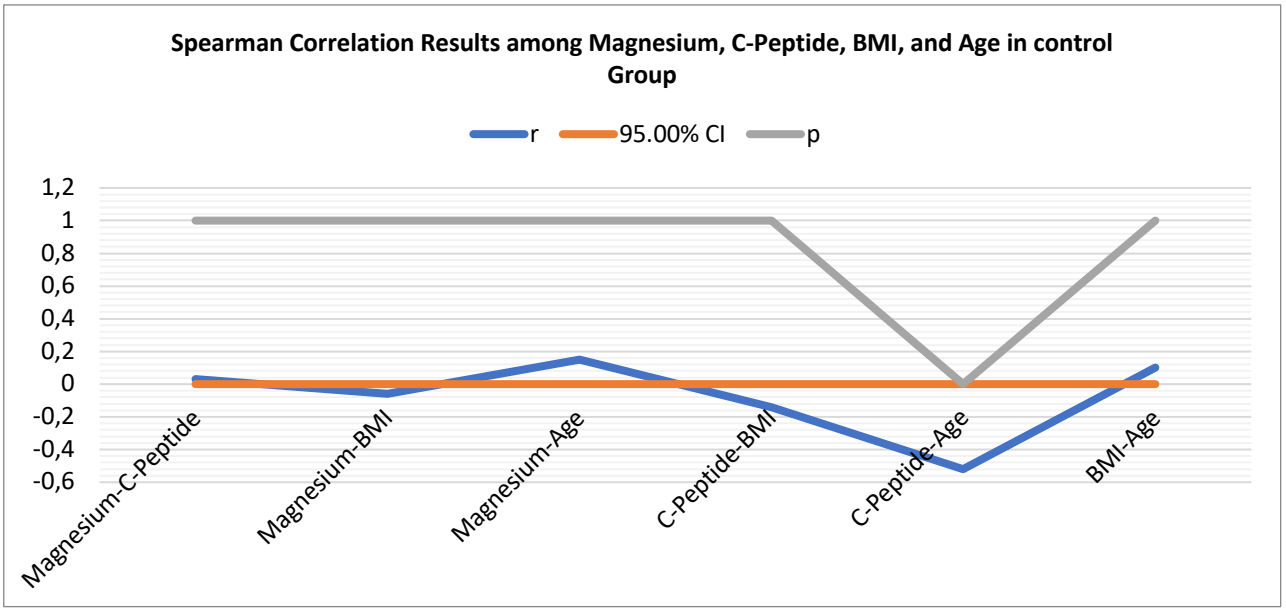

Fig. 6. Spearman Correlation Results in control Group

3.4. ROC curve characteristics of the study parameters as they classify PCOS from control subjects

The ROC curve characteristics of the study demonstrate its ability to classify polycystic ovary syndrome (PCOS) cases from controls, with magnesium levels having an area under the curve (AUC) of 0.803 with a standard error (SE) of 0.0352 and a 95% confidence interval extending from 0.733 to 0.862. The p value was less than 0.001, Cutoff criterion ≤1.184, and showed a sensitivity of 81.25% and a specificity of 73.75%. In contrast, C-PEPTIDE levels showed a higher AUC value of 0.875 with a SE of 0.0286, a 95% confidence interval between 0.814 and 0.922, and a p value of less than 0.001, and the criterion was set at (Cutoff) >2.592, with a sensitivity of 87.50% and a specificity reported at 80.00. %. These results indicate a high efficiency of these indicators in distinguishing between the study group and the control group regarding polycystic ovary syndrome (Table 4, Fig. 7 and 8).

Table 4. ROC curve characteristics of the study parameters as they classify PCOS from control subjects

Variable	AUC	SE	95% CI	p	cutoff	Sensitivity	Specificity
Magnesium	0.803	0.0352	0.733 to 0.862	<0.001	≤1.184	81.25	73.75
C-peptide	0.875	0.0286	0.814 to 0.922	<0.001	>2.592	87.50	80.00

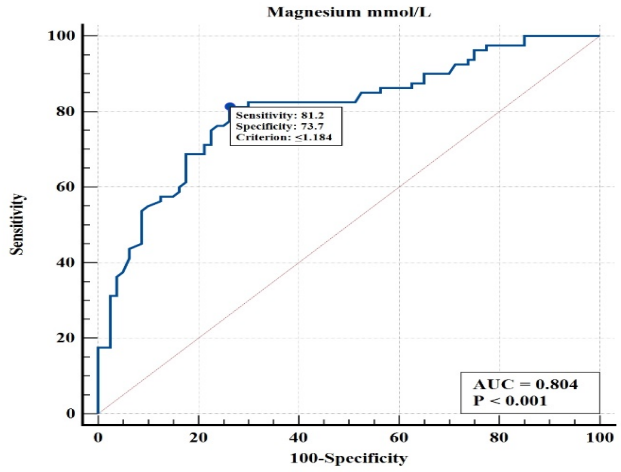

Fig. 7. ROC curve analysis of Mg as it differentiates between PCOS from control groups

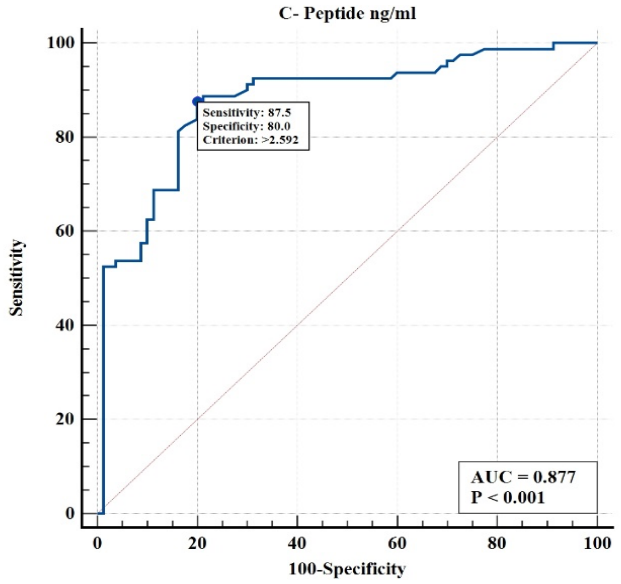

Fig. 8. ROC curve analysis of C-peptide as it differentiates between PCOS from control groups

4. Discussion

In this study, the serum level of C-peptide was significantly higher in PCOS patients compared to control group. This study agrees with a study done by Momo et al. (18), in which women with PCOS had a high C-peptide level and HOMA-IR index compared to matched controls suggesting greater insulin resistance. As anticipated, the PCOS group exhibited significantly higher C-peptide levels, which are indicative of insulin production, compared to the control group. This suggests the presence of hyperinsulinemia in this particular population. Women with PCOS exhibit elevated rates of basal insulin secretion. On the other hand, when faced with a high amount of glucose, the body's ability to produce insulin is usually not enough, resulting in a lower glucose disposition index compared to individuals of the same age and BMI who do not have this issue (19). Polycystic ovarian syndrome (PCOS) is primarily characterized by the presence of hyperandrogenism, hyperinsulinemia, obesity, and insulin resistance (IR). These manifestations are driven by many pathogenic pathways. A multitude of infrared markers have been assessed. The connecting peptide (Pep-C) is secreted in an equimolar ratio with insulin from pancreatic beta cells straight into the portal circulation. It plays a role in the folding, assembly, and processing of insulin. Additionally, it can serve as an alternate marker for insulin resistance (IR) and malfunction of beta cells. An increase in C-peptide levels indicates insulin resistance because it is considered important in the diagnosis for various reasons, including lack of metabolism in the liver and a longer period of work or life (5). In this study, it was found that magnesium levels were lower in the case group compared to the control group, and these results are consistent with Babapour et al. (20), The results also showed that magnesium levels in women with obesity, diabetes, and polycystic ovary syndrome were lower than those in thin women from the same group. This indicates the important role of magnesium in obesity and diabetes and its relationship with inflammatory conditions that can affect its levels in the blood. Furthermore, our understanding of the therapeutic potential of magnesium in treating endocrinological illnesses is constantly evolving. Rajeswari et al. (21) suggest that a deficiency in magnesium can lead to various biochemical issues associated with the reproductive abnormalities of PCOS. A separate investigation conducted by Rasoul et al. (22) reveals that individuals diagnosed with PCOS

exhibit significantly reduced levels of magnesium in their bloodstream. However, they also demonstrate elevated levels of magnesium in their urine when insulin is present. The findings of this randomized clinical trial indicate that administering a daily dose of 250 mg magnesium to women with PCOS did not yield substantial improvements in their hyperandrogenism, hirsutism, or sleep quality. The global prevalence of individuals consuming dietary supplements has been steadily increasing (21). However, it is important to note that many supplements may not confer any health benefits and could potentially have enduring adverse consequences (22). Consequently, it would be advantageous for study to examine the efficacy of dietary supplements in treating various conditions, such as PCOS (21, 22). In both the PCOS group and the control group, there was no correlation seen between the levels of magnesium in the serum and either C-peptide or body mass index (BMI). There is no statistical significance in magnesium levels, fat levels, insulin sensitivity, and sugar levels in women of reproductive age who suffer from polycystic ovary syndrome, and this is what we found Kaufmann and colleagues (14). This confirms the data we have found and the results shown Marj et al. (23) Magnesium supplements do not have a significant effect on lipid levels and blood sugar levels in women with polycystic ovary syndrome. On the contrary, according to other results, the levels of magnesium and C-peptide were under the curves of 0.803 and 0.875. By examining the deterministic values and the sensitivity and specificity ratios, this confirms Accuracy of results and levels reached in the research. A subsequent limited investigation revealed that even lean individuals with PCOS exhibit insulin resistance, so bolstering the concept that insulin resistance may be a core aspect of PCOS. Despite this finding, the existing diagnostic criteria for polycystic ovary syndrome (PCOS) continued to prioritize hyperandrogenism and ovarian morphology and function, while disregarding the clinical factors related to glucose metabolism. Consequently, there is a significant variation in metabolic diversity among the four phenotypes based on the diagnostic criteria established in Rotterdam. Within the subset of patients with phenotype A, insulin resistance (IR) is the most commonly proposed cause (24). According to recent estimates, 75% of women with polycystic ovarian syndrome exhibit insulin resistance as determined by the hyperinsulinemic-euglycemic clamp technique. Despite the considerable amount of research investigating the connection between obesity, decreased insulin sensitivity, the exact arrangement of this complex and mutually interdependent series of events, including hyperinsulinemia in women with polycystic ovarian syndrome, remains incomprehensible. The frequency of different assessments for insulin resistance (IR) and the absence of definitive evidence about the degree of IR in thin PCOS patients compared to age and BMI-matched controls (25) contribute to the increased level of uncertainty. In polycystic ovarian syndrome (PCOS), a hypothesis similar to that of type 2 diabetes suggests that the series of events begins with compensatory hyperinsulinemia in response to insulin resistance (IR), which temporarily maintains normal blood sugar levels. In individuals with a genetic predisposition, the presence of either a relative or absolute lack of insulin becomes evident as prediabetes or type 2 diabetes progresses (26). During this stage, a substantial part of the illness progression is marked by high levels of insulin in the blood, which directly and indirectly disturb ovarian function, while also affecting unexplained changes (9). Insulin can act as a co-gonadotrophin alongside luteinizing hormone in ovarian theca cells to promote the production of androgens. In addition, insulin inhibits pre-antral folliculation and supports the growth of follicles (27).

Patients with polycystic ovary syndrome (PCOS) showed a drop in serum magnesium levels and an increase in C-peptide levels. However, no statistically significant link was observed between these two variables. Further investigation and therapy are required to ascertain the role of magnesium and its correlation with PCOS, as a result of the reduced levels of magnesium in the serum of women with PCOS.

Ethical Statement

Ethical approval was received from the ethical and research committee of College of Medicine-Al-Nahrain University-Iraq. Informed consent was obtained from all participants (Decision No: 20211048, Date: 9/12/2021).

Conflict of interest

The authors declare there is no conflict of interest.

Funding

No funds were received to fulfill this work.

Acknowledgments

We would like to thank all the women who agreed to participate in this study, also we are most grateful to the laboratory staff and technicians of the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University and Al-Immamain Al-Kadhmain Medical city for their help in the analysis of the parameters in this study.

Authors' contributions

Concept: N.S.H., W.T.A., E.A.A.K., Design: N.S.H., W.T.A., E.A.A.K., Data Collection or Processing: N.S.H., W.T.A., E.A.A.K., Analysis or Interpretation: N.S.H., W.T.A., E.A.A.K., Literature Search: N.S.H., W.T.A., E.A.A.K., Writing: N.S.H., W.T.A., E.A.A.K.

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