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Using Serum Levels of Calcium, Magnesium, and Calcium/Magnesium Ratio to Predict Preeclampsia

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ABSTRACT

Background: Preeclampsia (PE) is a systemic disorder of pregnancy that is associated with significant perinatal morbidity and mortality. Calcium (Ca) and magnesium (Mg) play a pivotal role in regulating the contractility and tone of the cardiac and vascular muscle fibers.
Objectives: To assess the role of serum levels of Ca, Mg and Ca/Mg ratio as predictors of PE severity.
Materials and methods: A prospective cohort study was conducted that included 200 pregnant women who attended three prenatal checkups at 11, 24, and 35 weeks of gestation. Serum Ca and Mg levels were measured at each visit.
Results: At the 11th week, the mean serum Ca level, Mg level, and Ca/Mg ratio were 9.09 ± 0.60 mg/dl, 2.92 ± 0.72 mg/dl and 3.28 ± 0.76, respectively. At the 24th week, women with early PE had the lowest mean serum Ca (7.94 ± 0.64 mg/dl) as compared to those without the health problem (9.12 ± 0.60 mg/dl). At the 35th week, preeclamptic women had significantly reduced serum Ca, Mg, and Ca/Mg ratio compared to non-preeclamptic women. The lowest mean serum levels of Ca, Mg, and Ca/Mg ratio were found in severe compared to mild PE (P-value < 0.05). At the 11th week, the cutoff values of 8.18 mg/dl for serum Ca, 2.04 mg/dl for Mg, and 3.09 for Ca/Mg ratio were valuable for predicting the severity of PE at the 35th week of pregnancy with 100% sensitivity and a specificity of 87%, 91% and 81%, respectively.
Conclusion: The serum Ca, Mg, and the Ca/Mg ratio, measured at the 11th week of gestation, were critical predictors of severe PE at the 35th week.
Keywords: Calcium; Magnesium; Calcium/Calcium ratio; Severe preeclampsia.

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INTRODUCTION

Preeclampsia (PE) is a systemic disorder of pregnancy that is associated with significant perinatal morbidity and mortality and has the potential to result in maternal death worldwide, with a predominance in underdeveloped nations [1]. It presents clinically as new-onset hypertension with significant proteinuria that arises after 20 weeks of gestation. It represents a major public health concern, as approximately 5% of all expectant mothers will experience PE in the second half of their first pregnancy [2, 3]. A history of diabetes, multiple gestations, nulliparity, increased maternal age (≥ 40 years), and a previous history of preeclampsia have all been linked to an increased risk of PE [4].

While the exact origin of preeclampsia is still unknown, several ideas propose aberrant trophoblastic invasion and placental implantation as potential culprits [2]. This results in an inadequate adaptation of the maternal spiral arterioles, which can cause elevated vascular resistance in the uterine artery and reduced blood flow to the placenta. Moreover, the condition is characterized by multi-systemic manifestations and complications indicating cerebral damage, renal and hepatic impairment, and respiratory compromise [5]. It is believed that these symptoms are caused by widespread endothelial activation and dysfunction [2], and according to available data, endothelial dysfunction in preeclamptic pregnant women may continue for years after the event, increasing the likelihood

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that these women would develop cardiovascular problems in the future [6]. Hence, the development of strategies to predict and prevent this disorder has been both mandatory and challenging.

Although the literature has not yet addressed the condition's molecular cause [7], preeclampsia-related increased blood pressure has been hypothesized to be caused by variations in maternal serum ions [8]. Calcium (Ca) and magnesium (Mg) play a pivotal role in regulating the contractility and tone of the cardiac and vascular muscle fibers. It has been demonstrated that dietary deficiencies in mineral ions might affect developing fetuses and pregnant mothers, perhaps exacerbating PE [9, 10]. Although researchers have not shown evidence to support the routine administration of Mg for all pregnant women [11]; nonetheless, the majority of studies have documented lower Mg levels throughout pregnancy and much lower levels in PE [12, 13]. Additionally, several studies demonstrated that preeclamptic women exhibit a lower blood Ca level compared to healthy pregnant women [10, 14, 15].

Despite advances in prenatal care, identifying women at risk remains a challenge due to the complex and multifaceted nature of PE, which can present in a variety of ways. Adding to the complexity, the natural fluctuations in serum Ca, Mg, and their ratio during pregnancy, influenced by normal physiological changes like hemodilution as well as factors like diet and environment, making it tricky to differentiate between typical pregnancy variations and early warning signs of the condition [16]. Although the association of PE with Ca and Mg has been widely investigated [10, 12–14]. There is still limited data addressing the relation of the Ca/Mg ratio with the risk of the disease [12, 17]. Furthermore, there is little information regarding their role in predicting the severity of the condition. The main challenge in this study was developing a dependable way to predict severe PE early using serum Ca, Mg, and their ratio. Another significant hurdle was determining accurate cutoff values for Ca, Mg, and the Ca/Mg ratio. These thresholds needed to be precise, practical, and capable of predicting severe PE with high accuracy, ensuring their relevance and usefulness in clinical practice. Hence, we conducted this study on a group of women in early pregnancy at Kirkuk Governorate, Iraq, to test the involvement of Ca, Mg and Ca/Mg ratio in the risk of PE development and their relation with the severity of the disease.

MATERIALS AND METHODS

This prospective cohort study was conducted in Kirkuk City (Azadi Teaching Hospital) from the first of January 2024, to the end of September 2024. Women were enrolled at the three trimester antenatal visits (the mean gestational age at the first visit was 11 weeks, at the second visit was 24 weeks, and at the third visit was 35 weeks). Gestational age was calculated from the participants' menstrual cycle dates and confirmed by the first visit ultrasound.

The Ethical Approval Committee of the University of Kirkuk, College of Medicine, approved the current study (Reference number 59 dated 2024). Informed consent was obtained from all participants before their inclusion in the study.

Standard clinical data (e.g., maternal weight, and blood pressure) were collected at all research visits, and maternal weight and height ascertained at the time of the initial visit were used to measure body mass index (BMI) according to the equation BMI = weight (kg)/Height per m^2, with BMI $\leq$ 24.9 regarded as normal, 25–29.9 regarded as overweight, and $\geq$ 30 as obese.

Pregnant women with the following criteria were excluded from the current study: Majer fetal abnormalities (including a hydatidiform mole), multiple pregnancies and intrauterine fetal death, chronic hypertension, diabetes mellitus, chronic kidney disease, liver disease, cardiovascular diseases, hematological disorders, malignancy, cigarette smoking, taking Ca and Mg supplementation or using drugs that could influence their blood pressure, and those who lost to follow-up or were terminated before 35 weeks. Blood pressure was measured using the auscultatory method when the woman was at rest and seated. PE was classified according to its severity into two mild and severe. A blood pressure $\geq$ 140/90 mm mercury on two separate occasions at least 4 hours apart, and an amount of protein above 300 mg in a 24-hour urine collection or $\geq$ 1+ on a dip-stick test was considered as mild PE. Furthermore, severe PE was diagnosed when a pregnant woman meets one or more of the following criteria: Blood pressure of at least 160/110 mm mercury with proteinuria of at least 2+ on dip-stick testing, urine output of less than 500 ml per 24 hours, experiencing cerebral confusion, seizures or visual disturbance, having pulmonary edema, experiencing epigastric or right hypochondrial pain, having impaired hepatic function, thrombocytopenia, or fetal growth restriction [5].

Blood samples were obtained from the pregnant women at each visit to determine the quantities of serum Ca and Mg. Care was taken during blood collection to avoid using anticoagulants such as heparin, ethylenediaminetetraacetic acid, citrate, and oxalate. Serum Ca and Mg levels were measured by a spectrophotometric method using a SpinX automatic analyzer, from Spinreact/Spain. The reference ranges of Ca are 8.6–10.2 mg/dl and for Mg, 1.6–2.6 mg/dl.

Using Epi-Info, the sample size was calculated based on an estimated prevalence of PE of 4.79% [18]. We found that a sample size of 195 pregnant women was sufficient to achieve a 95% confidence interval for the prevalence with ± 3% precision. The study included 230 pregnant women carrying a single viable fetus, taking into account a 20% dropout rate.

Computerized statistical analysis was done using the Minitab version 23 program. The distribution of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests, which demonstrated that the data were normally distributed. Comparative analysis was performed using an independent t-test for comparison between continuous variables. Besides, the ANOVA test was used to compare the means of three variables. The Receiver operating characteristic (ROC) curve was performed to determine the cutoff values of Ca, Mg, and Ca/Mg ratio in the prediction of severe PE. The P-value > 0.05 was considered to be a statistically significant difference.

RESULTS

Figure 1 demonstates the enrolled and excluded pregnant women in the present study.

Table 1 shows the demographics of the studied pregnant women at the first visit (11^{th} week of gestation). The average age was 29.66 years, the mean parity was 2.2, the mean gestational age was 11.34 weeks, and the average BMI was 25.88 kg/m^2. The mean serum Ca, Mg, and Ca/Mg ratio at the first visit on the 11^{th} week were 9.09 mg/dl, 2.92 mg/dl and 3.28, respectively.

During the second visit at the 24^{th} week of pregnancy, among the 200 pregnant women, 10 (5%) were identified as

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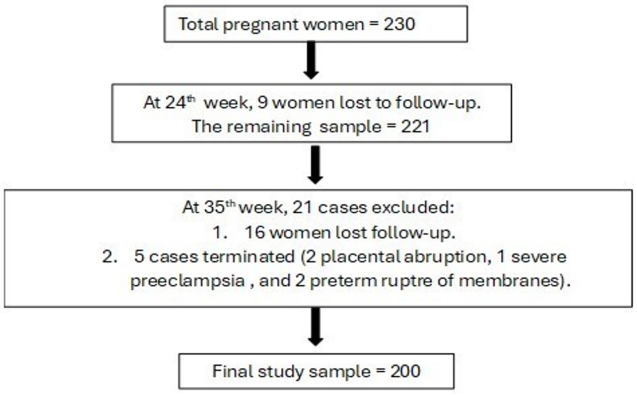

Figure 1. Flow chart of the 200 pregnant women.

Table 1. Basline characteristics of 200 pregnant women.

Variable	Pregnant women (n:200) (Mean ± SD)
Age (years)	29.66 ± 5.71
Parity	2.2 ± 1.3
Gestational age (weeks)	11.34 ± 2.7
Body mass index (kg/m^2)	25.88 ± 5.2
Serum calcium level (mg/dl)	9.09 ± 0.60
Serum magnesium level (mg/dl)	2.92 ± 0.72
Serum calcium/magnesium ratio	3.28 ± 0.76

having early PE. The study showed that women with PE had significantly (P-value < 0.05) higher mean and maximal values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) as shown in Table 2.

The study showed that the lowest mean of serum Ca (7.94 ± 0.64 mg/dl) was recorded among women with early PE (in the 2nd visit at 24th weeks of pregnancy) as compared with those without PE (9.12 ± 0.60 mg/dl) with a statistically

Table 2. Systolic and diastolic blood pressure of women with preeclampsia (PE) and non-PE pregnant women at the 2nd visit*.

Parameters	Pregnant women in in 24th week of gestation		P-value†
	PE(n:10) (Mean ± SD)	Non-PE(n:190) (Mean ± SD)	
Mean 24 hour SBP, mm Hg	138.6 ± 12.6	110.6 ± 5.3	0.011
Mean 24 hour DBP, mm Hg	95.7 ± 7.66	73.6 ± 5.2	0.021
Maximal SBP, mm Hg	167.4 ± 20.43	116.7 ± 7.66	0.018
Maximal DBP, mm Hg	122.4 ± 16.5	75.7 ± 9.5	0.017

* Independent sample t-test was used for the analysis, †Significant (P-value < 0.05), SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

significant difference (P-value < 0.05). While the serum Mg and Ca/Mg ratios did not differ significantly between the two groups in the same visit (Table 3).

In the third visit, at 35 weeks of gestation, 33 of the 200 women were found to have PE (16.5%). The study demonstrated that serum Ca, Mg, and the Ca/Mg ratio (7.09 ± 0.68 mg/dl, 2.33 ± .047 mg/dl, and 3.42 ± 0.64, respectively) were significantly (P-value < 0.05) reduced in pregnant women with PE within the 35th week of pregnancy as compared with non-PE pregnant women in the same period (Table 4).

Among the 33 PE women in the 35th week of pregnancy, 11 patients exhibited evidence of severe PE, while the remaining 22 had mild severity.

The results revealed a significant progressive increase in systolic and diastolic blood pressure readings from the group without PE to those in the other two groups (with mild and severe PE). For instance, the mean 24-hour systolic blood pressure (SBP) rises from 113.6 ± 4.6 mm Hg in the non-PE group to 151.2 ± 5.1 mm Hg in mild PE and 171.8 ± 6.4 mm Hg in severe PE (P-value = 0.001). Similarly, the mean 24-hour diastolic blood pressure (DBP) and maximal blood pressure readings follow a comparable pattern, with the highest values observed in the severe PE group (Table 5).

The lowest mean serum levels of Ca (7.76 ± 0.69 mg/dl), Mg (2.34 ± 0.65 mg/dl), and the Ca/Mg ratio (3.31 ± 0.63) were found in women with severe cases of PE compared to those with mild PE. These differences were statistically significant (P-value < 0.05) as shown in Table 6.

The study found that serum Ca, Mg, and Ca/Mg ratio, measured at the 11th week of gestation, are critical predictors of severe PE by the 35th week. For Serum Ca, the cutoff of value 8.18, with a sensitivity of 100% and a specificity of 87%, is suggested as a strong predictor of severe PE. Serum Mg has a cutoff value of 2.64, with a sensitivity and specificity of 100% and 91%, respectively, also showing excellent discriminative power. For the Ca/Mg ratio, the cutoff is 3.09, with sensitivity of 100% and specificity of 81%, suggesting moderate accuracy. All P-values are significant (P-value < 0.001), supporting the reliability of these findings (Figure 2).

DISCUSSION

PE is a prevalent factor contributing to health issues and deaths among both mothers and fetuses [6]. Nevertheless, the precise cause of PE remains unidentified. Several studies have inspected the relationship between PE and the fluctuation in the blood electrolytes, namely Ca and Mg, with inconsistent findings [9, 19, 20]. The distinctive value of this study lies in its prospective design, which allowed for systematic monitoring of women from early pregnancy through the second and third trimesters. This approach provided valuable longitudinal data on serum Ca and Mg, as well as their ratio, about the development and severity of PE. Furthermore, the study's focus on assessing the predictive efficacy of these parameters during the first trimester underscores its contribution to advancing early diagnostic strategies for severe PE. By addressing a critical gap in prenatal care, this study offers practical insights for improving maternal and fetal outcomes.

The study revealed that women who had early PE exhibited significantly lower serum Ca levels during the 24th week of pregnancy, in comparison to those who did not. In the instances of early PE, although lower average levels of Mg, and Ca/Mg ratio were observed, it did not reach statistical sig-

Table 3. Comparison of serum calcium (Ca), magnesium (Mg) levels, and Ca/Mg ratio in pregnant women with and without preclampsia (PE) at 24th weeks of gestation*.

PE in the 24th week of gestation	No.	Serum Ca level (mg/dl) Mean ± SD	Serum Mg level (mg/dl) Mean ± SD	Serum Ca/Mg ratio Mean ± SD
Yes	10	7.94 ± 0.64	2.37 ± 0.41	3.44 ± 0.61
No	190	9.12 ± 0.60	2.57 ± 0.52	3.68 ± 0.72
P-value		0.001†	0.11	0.16

* Independent sample *t*-test was used, †Significant (P-value < 0.05).

Table 4. Serum calcium (Ca) and magnesium (Mg) levels, and their ratio between pregnant women with and without preeclampsia (PE) at the 35th week of gestation*.

PE in the 35th week of gestation	No.	Serum Ca level (mg/dl) Mean ± SD	Serum Mg level (mg/dl) Mean ± SD	Serum Ca/Mg ratio Mean ± SD
Yes	33	7.90 ± 0.68	2.33 ± 0.47	3.42 ± 0.64
No	167	9.09 ± 0.64	2.51 ± 0.49	3.56 ± 0.68
P-value†		0.001	0.001	0.01

* Independent sample *t*-test was used, †Significant (P-value < 0.05).

Table 5. Comparison of blood pressure parameters among pregnant women at the 35th week of gestation according to the presence and severity of preeclampsia (PE)*.

Parameters	Pregnant women at 35th of gestation			P-value†
	Without PE	Mild PE	Severe PE	
Mean 24 hour SBP, mm Hg	113.6 ± 4.6	151.2 ± 5.1	171.8 ± 6.4	0.001
Mean 24 hour DBP, mm Hg	76.7 ± 8.2	94.5 ± 9.1	119.3 ± 10.2	0.001
Maximal SBP, mm Hg	130.5 ± 7.7	156.6 ± 8.2	185.4 ± 9.0	0.001
Maximal DBP, mm Hg	83.5 ± 8.2	108.1 ± 7.9	132.2 ± 8.6	0.001

* ANOVA test, †Significant (P-value < 0.05). SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 6. Comparison of serum calcium (Ca) and magnesium (Mg) levels, and Ca/Mg ratio in pregnant women with mild and severe preeclampsia (PE) at 35th weeks of gestation*.

Severity of PE	No.	Serum Ca Level (mg/dl)	Serum Mg Level (mg/dl)	Serum Ca/Mg Ratio
Mild	22	7.99 ± 0.67	2.36 ± 0.40	3.47 ± 0.65
Severe	11	7.76 ± 0.69	2.34 ± 0.65	3.31 ± 0.63
P-value†		0.015	0.038	0.041

* Independent sample *t*-test, †Significant (P-value < 0.05).

nificance. The serum Ca level drops throughout the 2nd and 3rd trimesters of pregnancy, mostly because of hemodilution [10]. Extremely low serum Ca levels may be linked to certain problems of pregnancy, such as PE, low birth weight, premature delivery, and neonatal death [12, 19]. Abnormally low Ca levels may stimulate parathyroid hormone secretion and release renin, thereby inducing vasoconstriction and causing an elevation in blood pressure [9]. Our findings regarding Ca are consistent with the findings of other studies [10, 14], while they contradict the study of Wadhwani et al. [13] in which the Ca level was similar between the two groups of PE and controls. Okoror et al. [12] conducted a study on the correlation of serum Ca, Mg and Ca/Mg ratio with PE. They discovered that preeclamptic women had significantly lower levels of serum Ca, Mg, and Ca/Mg ratio compared to normotensive women. However, their study was conducted on women at later gestational age and does not include women with early-onset PE specifically.

In this study, at 35 weeks of gestation, 33 women (16.5%) developed PE, with an incidence rather higher compared to the worldwide estimates, which might be attributed to geographical and population-specific variables [2, 5]. The study found that pregnant women with PE experienced significantly reduced serum Ca, Mg, and Ca/Mg ratios within the 35th week of pregnancy compared to non-preeclamptic women. Ca and Mg ions are essential for maintaining the cell membrane

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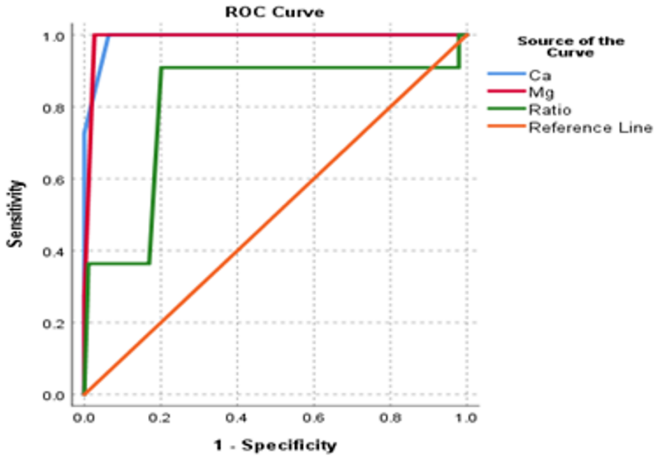

Figure 2. Cutoff values of serum calcium (Ca), magnesium (Mg), and (Ca/Mg) ratio in the prediction of severe preeclampsia.

potential in muscles and nerves [14]. During cellular injury, the intracellular influx of Ca increases its intracellular concentration and stimulates the compensatory intracellular influx of Mg, its physiological antagonist, leading to the loss of homeostasis of both ions [17]. This may explain the initial low levels of Ca at 24 weeks, followed by the lower levels of both ions, as well as lower Ca/Mg ratios observed later on at 35 weeks. However, there is conflicting evidence in the literature on the involvement of both ions and their ratio in the pathogenesis of PE. Our findings regarding Ca and Mg are consistent with other research [15, 21]. Additionally, Okoror et al [12] and Winarno et al. [17] pointed out that having lower Ca/Mg ratios could mean a higher risk of developing PE, since both Ca and Mg are important for keeping blood vessels healthy and functioning properly. On the other hand, Darkwa et al. [20] discovered that there was no disparity in Ca and Mg levels between women with PE and those who did not. Studies by Tuli et al. [22] and Roy et al. [23] revealed a higher average Ca level in the PE group compared to the non-PE group. These differences can be related to variations in dietary intake between study populations.

Additionally, findings from the current study revealed significantly lower levels of Ca and Mg and lower Ca/Mg ratios in the group of patients who were diagnosed with severe PE in comparison to mild cases. This was consistent with findings from other studies [16, 19]. Typically, Mg is recognized as a crucial component for numerous enzyme systems, and it has a significant impact on the transmission of neurochemicals and the widening of blood vessels in the peripheral nervous system [17]. Furthermore, Ca and Mg compete with each other at presynaptic junctions. A reduction in the ratio of Ca to Mg in the bloodstream can heighten excitability and lead to burst firing, long-term potentiation, pain transmission, epileptogenesis, nerve injury, and eventually precipitate eclamptic convulsions in severe PE [17].

Interestingly, the current study revealed that serum Ca, Mg, and the Ca/Mg ratio measured at the 11th week of gestation strongly predicted severe PE by the 35th week. At specific levels (8.18 mg/dl for Ca and 2.64 mg/dl for Mg), serum Ca and Mg were very good at identifying severe PE, making them a strong option for early testing and detection during pregnancy. This finding is in line with previous research that has linked low serum Ca and Mg levels to an elevated risk of severe PE [16, 19]. In a study by Winarno et al. [17], the level of serum Ca that could predict PE was found to be 4.65 mg/dl, with a sensitivity of 60.2% and specificity of 52.9%. This difference in the cutoff level of Ca and its diagnostic value compared to our findings may be related to the difference in the study design, as it was assigning PE in general, and population differences. Surprisingly, the mean Ca levels in both groups of PE and healthy pregnancy in that study were much below the standard ranges of adult women. Contrarily, Gabbay et al. [24] found that hypocalcemia during the first trimester is not a risk factor for predicting PE.

Highlighted with a cutoff value of 3.09, the Ca/Mg ratio was another strong indicator of severe PE in the present study due to its high sensitivity and specificity. This finding corroborates the idea that the Ca/Mg ratio can serve as a robust indicator of severe PE. The ability to predict severe PE as early as the 11th week of gestation creates a critical window of timely interventions, potentially improving outcomes for both the mother and baby. Similarly, Winarno et al study [17] at a cutoff level of 2.36 Ca/Mg ratio was able to predict PE with a sensitivity of 46.8% and specificity of 62.3%.

However, one of the limitations of the current study was that the evaluation of the patients' intake of Ca and Mg was not conducted in the current study. Hence, it is unclear if the detected low levels of these minerals were merely related to the disease or there is a contribution from insufficient consumption.

CONCLUSION

This study revealed that there were lower levels of serum Ca, Mg, and Ca/Mg ratio in association with PE. Furthermore, the serum Ca, Mg, and the Ca/Mg ratio, measured at the 11th week of gestation, were critical predictors of severe PE at the 35th week. Our findings shed light on the benefits of utilizing a compound prediction model through early prenatal screening of serum Ca and Mg coupled with periodic monitoring of the Ca/Mg ratio throughout pregnancy.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

The Ethical Approval Committee of the University of Kirkuk, College of Medicine approved the current study (Reference number 59 dated 2024). All participants gave informed consent to be enrolled in the study.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

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