Italian Journal of
Gynæcology & Obstetrics
September 2023 - Vol. 35 - N. 3 - Quarterly - ISSN 2385 - 0868

Evaluation of haemoglobin and serum ferritin level in preterm labour and preterm prelabour rupture of membrane (PPROM): a case-control study

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ARTICLE INFO

History
Received: 21 October 2022
Received in revised form: 19 November 2022
Accepted: 14 December 2022
Available online: 20 September 2023

DOI: 10.36129/jog.2022.90

Key words
Preterm labour; anaemia; ferritin; PPROM.

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ABSTRACT

Objective. Evaluate the level of maternal haemoglobin and ferritin in preterm labour and preterm prelabour rupture of membrane (PPROM) among Iraqi women.
Materials and Methods. The case-control study conducted at Azadi Teaching Hospital-Kirkuk included 225 pregnant women equally distributed into three groups, preterm, PPROM, and control group. A maternal blood sample (5 ml) was collected at the time of presentation, haemoglobin and ferritin measurements were performed for all participants.
Results. Haemoglobin showed a significantly low level among the preterm and PPROM groups compared to control group ($p = 0.0001$). While ferritin showed a significantly low level among the preterm group compared to other groups ($p = 0.0001$). Comparing preterm with control, a haemoglobin level of ≥ 10 was associated with 89% sensitivity and 44% specificity, and a ferritin level of ≥ 32 was associated with 84% sensitivity and 61% specificity. Comparing PPROM with control, a haemoglobin level of ≥ 10 was associated with 80% sensitivity and 46% specificity, and a ferritin level of ≥ 74 was associated with 80% sensitivity and 46% specificity. In logistic regression analysis for preterm and control groups, only Hb was found to be a significant independent risk factor for preterm and for PPROM control groups, both Hb and ferritin level were found to be significant independent risk factors for PPROM.
Conclusions. Pregnant women with anaemia and a lower ferritin level are at risk of preterm labour, while those with anaemia and high level of ferritin are at risk of PPROM.

INTRODUCTION

Preterm labour is defined as regular uterine contractions before 37 completed weeks of gestation with intact membranes with 4 cm or more of cervical dilatation observable during 2 hours [1]. Preterm pregnancy accounts for approximately 10% of total pregnancies [2]. Iron deficiency is the most common cause of anaemia and is the most widespread nutritional disorder in the world [3, 4]. Anaemia had the potential risk of increasing preterm birth in addition to many other maternal diseases during and after pregnancy [5]. Since anaemia is the most common diagnosis among pregnant women [6], it could be significantly associated with preterm labour as well as other comorbidities [7].

Preterm premature rupture of membranes (PPROM) is defined as spontaneous rupture of foetal membranes before the onset of 37 completed weeks before labour and one of the main risks of PPROM is infection [8]. It occurs in 3% of pregnancies and is the cause of approximately one third of preterm deliveries. It can lead to significant perinatal morbidity and mortality [9].

The cause of preterm delivery can be multifactorial, there are several factors involved in the aetiology of preterm labour including maternal age, parity, level of education, pregnancy interval, previous history of miscarriage or preterm labour, antepartum haemorrhage, maternal hypertension, recurrent urinary tract infection, and some cases occur without apparent risk factors [10]. In Iraq, the main causes of preterm labour and PPROM were low socioeconomic status, heavy manual work, urinary tract infection, and previous obstetrical history. Iraq has been affected by wars and pregnant women are susceptible group and suffer from consequences of malnutrition, in addition to their exposure to stress and anxiety [11]. Ferritin is one of the acute phase reactants that increases with inflammation and could play an important role in identifying the PPROM that results from infection.

In response to the call for early detection of preterm labour, several diagnostic biomarkers are currently being developed. According to the main role of inflammation in the appearance and progression of preterm delivery, it is hypothesised that the measurement of serum ferritin level as a sensitive inflammatory marker can effectively predict this event in the high-risk group. Some investigators have reported a relationship between elevated serum ferritin concentrations and preterm labour [12].

Therefore, in this study, our aim is to assess the impact of anaemia and ferritin levels on preterm delivery and PPROM.

MATERIALS AND METHODS

A case-control study was carried out in the Department of Gynecology and Obstetrics of Azadi Teaching Hospital-Kirkuk, from 1st December 2018 to 1st April 2019. The study included 225 pregnant women with gestation ages between 24-36+6 days. Participants were informed about the nature of the study. The study ethics were implemented regarding the Helsinki Declaration by oral informed consent of the participants and agreement of the health authorities. Gestational age is calculated from the last menstrual period for those women with reliable menstrual cycles and confirmed by ultrasound assessment of foetal biometry that was done in gestational age less than 20 weeks. For those women with unreliable cycles or do not know their last menstrual period we depend on ultrasound measurement for foetal biometry at a gestational age of fewer than 20 weeks. The participants were divided into 3 groups:

- Group A: 75 pregnant with preterm labour and intact membrane.
- Group B: 75 pregnant with preterm prelabour rupture of membrane.
- Group C: 75 control normal pregnant women of the same gestational age attending the outpatient clinic or labour ward for antenatal care without uterine contraction or signs of labour.

Exclusion criteria include 1) maternal such as gestational and pregestational diabetes mellitus, thyroid disease, preeclampsia, eclampsia, chronic hypertension and heart disease, antepartum haemorrhage, urinary tract infection, acute febrile illness, chorioamnionitis, and smokers, 2) foetal such as foetal distress, intrauterine growth restriction, intrauterine foetal death, and foetal anomalies.

A detailed history was taken. The body mass index was measured for all groups.

A general and abdominal examination was performed. Preterm labour is determined by documented uterine contractions by cardiotocography (CTG) at least once every 10 minutes with an estimated cervical length of less than 1 cm and cervical dilatation of more than 2 cm, cervical effacement and dilatation were assessed by sterile speculum examination. Preterm prelabour rupture of membrane is

Table 1. *Demographic characteristics of participants.*

Variable	Preterm	PPROM	Control	Total	P-value
Age Mean ± SD (year)	30 ± 7	31 ± 4	30 ± 4	30.25 ± 7.5	
< 18 years	8 (10.7%)	2 (2.7%)	7 (9.3%)	17 (7.6%)	
19-25 years	13 (17.3%)	22 (29.3%)	17 (22.7%)	52 (23.1%)	
26-30 years	12 (16%)	13 (17.3%)	10 (13.3%)	35 (15.6%)	0.99*
31-36 years	24 (32%)	17 (22.7%)	18 (24%)	59 (26.2%)	
> 36 years	18 (24%)	21 (28%)	23 (30.7%)	62 (27.6%)	
Gravida					
1-3	37 (49.3%)	40 (53.3%)	29 (38.7%)	106 (47.1%)	0.28**
4-5	26 (34.7%)	19 (25.3%)	27 (36%)	72 (32%)	
≥ 6	12 (16%)	16 (21.3%)	19 (25.3%)	47 (20.9%)	
Parity					
0-2	36 (48%)	44 (58.7%)	37 (49.3%)	117 (52%)	
3-4	27 (36%)	18 (24%)	25 (33.3%)	70 (31.1%)	0.56**
≥ 5	12 (16%)	13 (17.3%)	13 (17.3%)	38 (16.9%)	
Gestational age (weeks) Mean ± SD	31 ± 3	31 ± 4	30 ± 4	30.75 ± 3.6	0.34*
Hb (g/dl) Mean ± SD	9.9 ± 1.4	9.7 ± 0.9	11.3 ± 0.9	10.2 ± 1.4	0.0001*
S.ferritin (ng/ml) Mean ± SD	43.3 ± 42 .8	90.9 ± 32	76.1 ± 41.1	70.1 ± 43.6	0.0001*
BMI (Kg/m²) Mean ± SD	30.2 ± 5.2	29.7 ± 3.9	30.2 ± 5.7	30 ± 4.9	0.78*

*ANOVA test; **chi-square test.

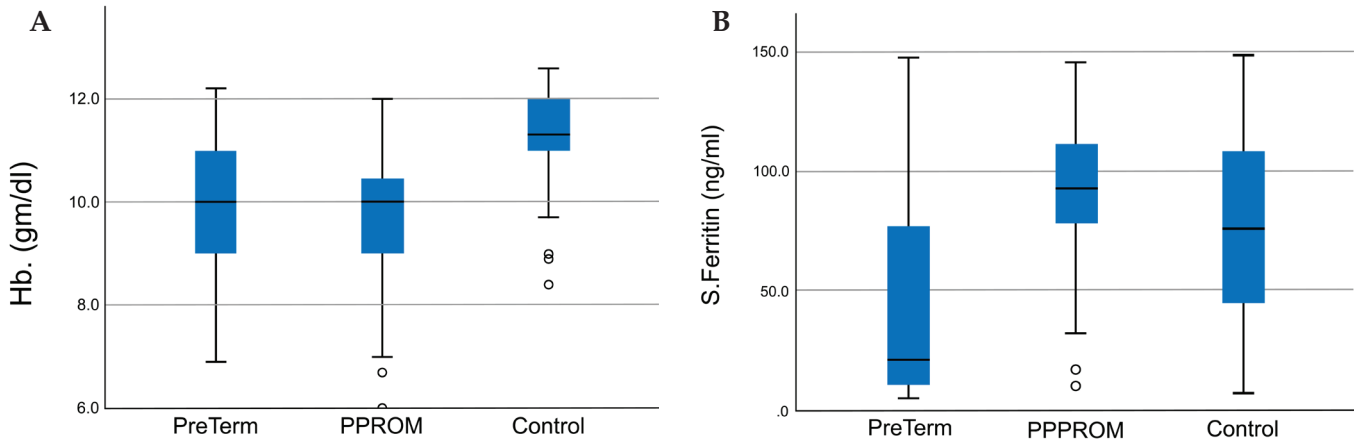

Figure 1. *Distribution of haemoglobin (**A**) and S. ferritin (**B**) across groups.*
*(**A**) Distribution of haemoglobin across groups (p = 0.0001). (**B**) Distribution of S. ferritin across groups (p = 0.0001). Independent-sample Kruskal-Wallis Test.*

confirmed by direct visualization of pooling of amniotic fluid during sterile speculum examination. Ultrasound evaluations for foetal biometry, foetal anomaly, and amniotic fluid index were performed for all groups. A maternal blood sample (5 ml) was collected at the time of presentation for all participants. Haemoglobin was determined by the colorimetric method. The sample is collected in EDTA k3 tubes (anticoagulated), then rolled in a KJMR2 roll mixer and then applied to the swelab haematology autoanalyzer.

Ferritin measurement

The sample is collected in a plane tube and then left for 20-30 minutes at room temperature for clotting; then the serum is separated by centrifugation at 4,000 RPM. Serum is applied to the Cobas e 411 device (Roche), which uses the electrochemiluminescence immunoassay method for the quantitative *in vitro* determination of ferritin in human serum. Normal range (women 17-60 years: 13-150 ng/ml).

Statistical analysis

The data was collected and sorted in Microsoft excel and is important for SPSS 24 and Stat 17. The mean and SD or count and percentage were presenting continuous and categorical variables, respectively. The ANOVA test was used to assess the comparison of continuous variables between three groups and the Chi-square was used for categorical variables. A receiver operating characteristic (ROC) curve is used to assess the sensitivity and specificity of the cut values.

RESULTS

There were 225 participants in this study who were divided for three groups, group A (preterm = 75), group B (PPROM = 75) and group C (control = 75).

Demographic characteristics

The mean age of the participants did not differ significantly between all groups, and more than half of the participants whose age were above 30 years old. Gravida and parity showed that nearly half of the participants were above gravida three and para two. The gestational age was the same for all participants as BMI which was also equal across three groups.
The haemoglobin showed a significantly low level among the preterm and PPROM groups compared to the control group (p = 0.0001), while ferritin showed a significantly low level among the preterm group compared to other groups (p = 0.0001) (**Table 1**). **Figure 1** shows the distribution of haemoglobin and ferritin among groups.
The gross correlation of haemoglobin across all participants with age, gestational age, BMI, and ferritin showed that only ferritin had a weak and significant positive correlation with haemoglobin, while other variables did not show a significant correlation (**Table 2**). In addition, ferritin showed only a significantly weak correlation with haemoglobin (**Table 3**).
Haemoglobin was lower for the preterm and PPROM groups compared to the control group across all age categories. The ferritin distribution showed different patterns of distribution in which it was lower for the preterm group in all age categories except the 26–30-year age group which showed the same level of ferritin in three groups in that age category. Interestingly, ferritin was higher in PPROM groups in all age categories in comparison to preterm and control groups (**Figure 2**).
ROC curve showed that comparing preterm with control cases, a haemoglobin level of ≥ 10 was associated with 89% sensitivity and 44% specificity (AUC 0.81, 95%CI 0.74-0.87) and a ferritin level of ≥ 32 associated with 84% sensitivity and 61% specificity (AUC 0.7135, 95%CI 0.62-0.79). Comparing PPROM with control cases, a haemoglobin level of ≥ 10 was associated with 80% sensitivity and 46% specificity (AUC 0.87, 95%CI 0.81-0.93), and a ferritin level of ≥ 74 was associated with 80% sensitivity and 46% specificity (AUC 0.61, 95%CI 0.51-0.7) (**Figure 3**).
When performing the binary logistic regression analysis for preterm and control groups (as reference group), only Hb was found to be a significant independent risk factor for preterm (OR 3.2, 95%CI 1.8-5.4) and for PPROM (as the reference group) groups, both Hb (OR 14.3, 95%CI 6-34.3) and the ferritin level (OR 0.9, 95%CI 0.94-0.97) were found to be significant independent risk factors for PPROM (**Table 4**).

DISCUSSION

Increasing the evidence of the negative impact of anaemia on poor maternal outcomes needs more justification in terms of a proper understanding of the impact and how to overcome it to decrease the likelihood of both preterm labour and PPROM. In this study, we found strong evidence of anaemia as a poor predictor factor for preterm labour and PPROM, this was consistent with a recently published study from Iraq that demonstrated the negative impact of anaemia on preterm birth [13], as well as to other global studies [14,15].

Table 2. *Person's correlation for haemoglobin.*

	Age (years)	Gestational age (weeks)	BMI (Kg/m^2)	S.ferritin (ng/ml)
		Hb (g/dl)		
Pearson correlation	0.017	0.012	0.104	0.321**
P-value	0.800	0.853	0.120	0.0001
n	225	225	225	225

Table 3. *Person's correlation for ferritin.*

	Hb (g/dl)	Age (years)	Gestational age (weeks)	BMI (Kg/m^2)
		S.ferritin (ng/ml)		
Pearson correlation	0.321**	-0.029	-0.127	0.002
P-value	0.0001	0.668	0.057	0.972
n	225	225	225	225

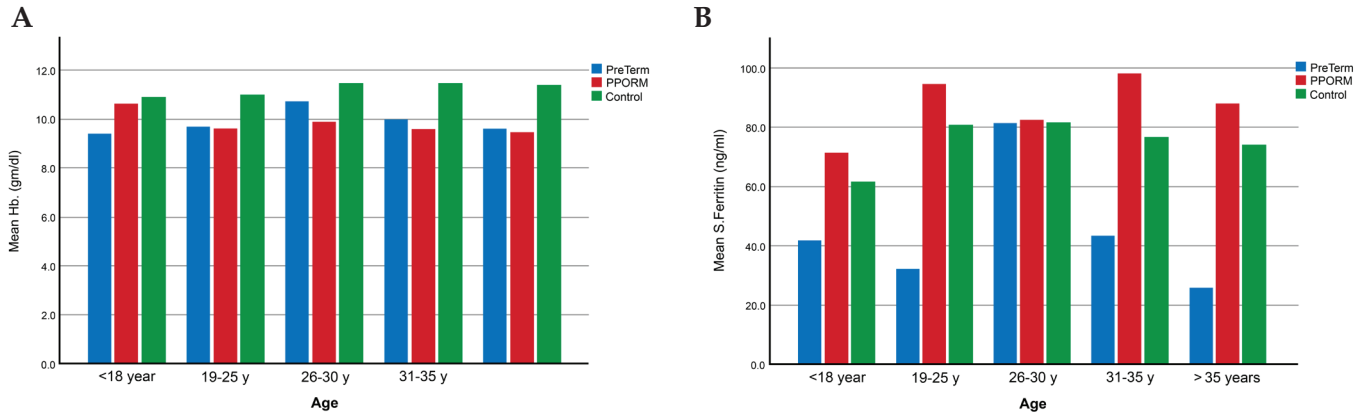

Figure 2. *Distribution of haemoglobin (**A**) and ferritin (**B**) between groups according to age categories.*

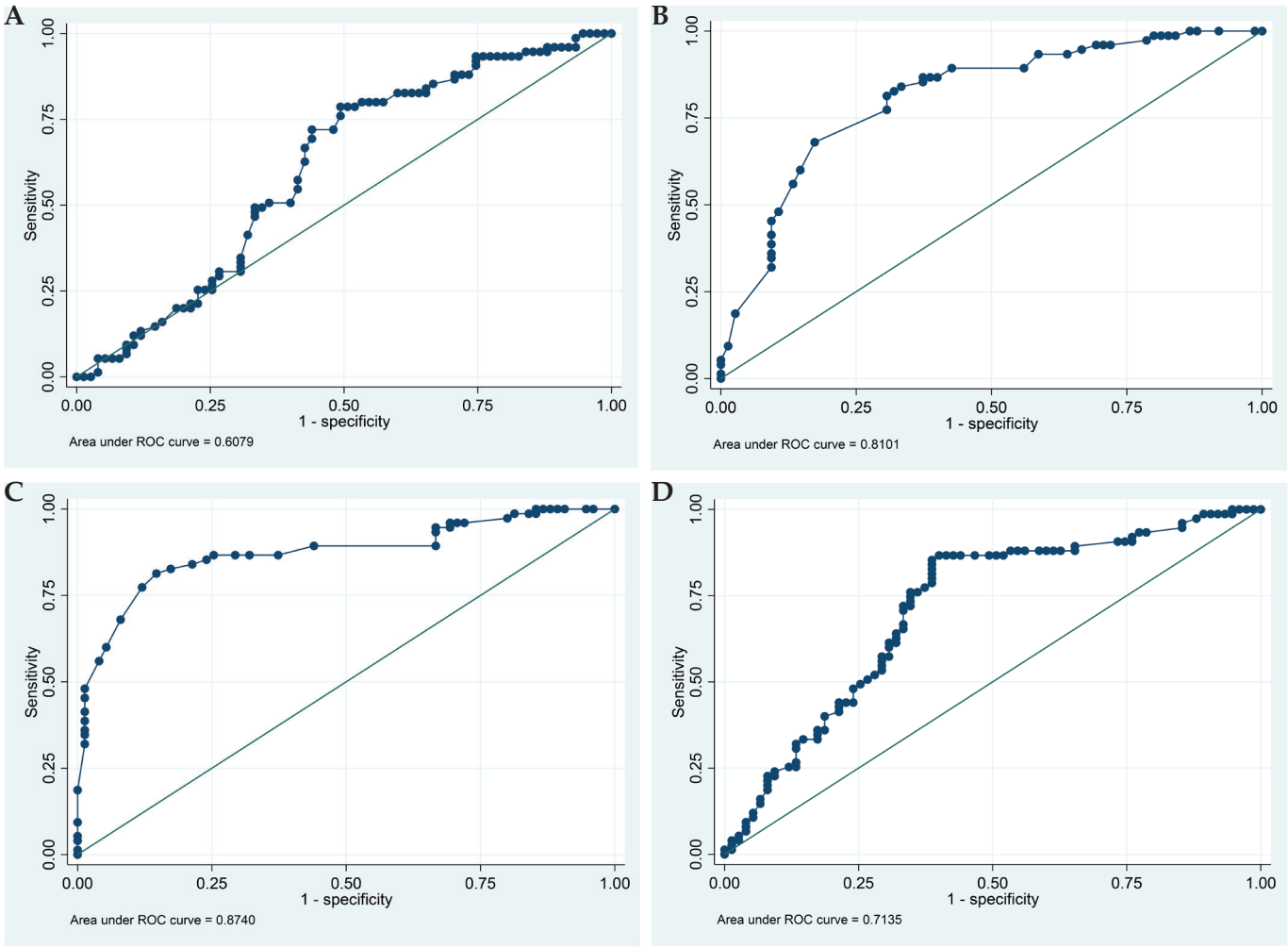

Figure 3. *A receiver operating characteristic comparing preterm to control (**B**,**D**) and PPROM to control (**A**,**C**), respectively.*

Also, because ferritin serves as an acute phase reactant, ferritin in this study showed a significantly higher level among the PPROM group compared to other groups.

This increases the evidence in the previously published literature that showed a strong association between ferritin level and PPROM [16, 17]. Furthermore, the increase in PPROM subsequently increased the preterm labour, and this could indirectly contribute the effect on ferritin level to increase the preterm labour [9].

Interestingly, the research by Zhang *et al*. showed that only anaemia in early pregnancy could contribute to an increased risk of preterm, while later anaemia or late exposure to anaemia in pregnancy did not [3]. This may be true since we did not as-

Table 4. *Binary logistic regression analysis for preterm and PPROM with control group.*

	P-value	OR	95%CI	
			Lower	Upper
Preterm				
Gestational age	0.690	0.979	0.880	1.088
Hb (g/dl)	0.0001	3.217	1.890	5.476
S.ferritin (ng/ml)	0.945	1.000	0.989	1.011
BMI (Kg/m^2)	0.269	0.960	0.893	1.032
PPROM				
Gestational age	0.015	0.839	0.729	0.966
Hb (g/dl)	0.0001	14.383	6.031	34.303
S.ferritin (ng/ml)	0.0001	0.958	0.941	0.977
BMI (Kg/m^2)	0.285	0.946	0.856	1.047

sess when anaemia started and how it evaluated in pregnancy with anaemia in early gestation.

In this study, the ferritin was significantly higher among PPROM compared to other groups and specifically to the preterm labour group. This is in line with a recent study from Iraq showing that serum ferritin was higher significantly among PPROM compared to preterm labour women [18]. This increased the evidence of an inflammatory base that led to PPROM, especially infection-based inflammation [19, 20]. In pregnancy, the change in pH level increases the vulnerability to bacterial infection [21]. In addition, in another study by Lamia, she demonstrated that high ferritin levels could be used as a predictor for the prediction of PPROM, as well as spontaneous preterm delivery [22].

Different levels of Hb and ferritin have been identified to predict preterm labour and PPROM, in this study we found that a haemoglobin level of ≥ 10 was associated with 89% sensitivity and 44% specificity, and ferritin level of ≥ 32 was associated with 84% sensitivity and 61% specificity. When comparing PPROM with control cases, a haemoglobin level of ≥ 10 was associated with 80% sensitivity and a 46% specificity, and ferritin level of ≥ 74 was associated with 80% sensitivity and 46% specificity. In a study by Abdel-Malek *et al*., they found that the cutoff value of serum ferritin of 31 ng / ml was associated with a sensitivity of 92.8% and specificity of 99.4% [16]. However, they used a small sample size for comparison between groups, which could impact negatively their results. Furthermore, the low-sensitivity results in this study could be due to the fact that both preterm and PPROM have many other causes that could interfere with Hb and ferritin. We identified that Hb was found to be a significant independent risk factor for preterm and PPROM, while ferritin is a significant independent risk factor for PPROM. This increases the previously mentioned evidence that ferritin is associated significantly with PPROM.

CONCLUSIONS

Pregnant women with anaemia and a lower ferritin level are at risk of preterm labour, while those with anaemia and high level of ferritin are at risk of PPROM.

Implications of these findings for clinical practice and/or future research

Haemoglobin level of ≥ 10 and a ferritin level of ≥ 32 could be proposed as a helpful marker that can predict preterm labour. While haemoglobin level of ≥ 10 and a ferritin level of ≥ 74 can be a helpful marker for prediction of PPROM.

Limitation of the study

We did not study the time of initiation of anaemia nor the correction status of anaemia whether the participants receive a treatment for preidentified anaemia or not. This could be important factors to be considered for future research.

COMPLIANCE WITH ETHICAL STANDARDS

Authors contribution

E.A.M.: Conceptualization, formal analysis, writing – original draft, writing – review & editing.

M.F.H.: Laboratory investigations, methodology, supervision, validation, resources.

Funding

None.

Study registration

The study has been registered in College of Medicine, University of Kirkuk.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

Ethical Committee of the University of Kirkuk, College of Medicine, approved the study.

Informed consent

The study ethics were implemented with regard to the Declaration of Helsinki by oral informed consent of the participants.

Data sharing

Data are available under reasonable request to the corresponding author.

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