Provisionally accepted for publication

ORIGINAL ARTICLE

Evaluation of Hemoglobin and Serum Ferritin level in Preterm Labour and Preterm Prelabour Rupture of Membrane (PPROM): a Case-Control Study

Short title: Hemoglobin and ferritin in preterm labour.

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Doi: 10.36129/jog.2022.90

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ABSTRACT

Objective. Evaluate the level of maternal hemoglobin and ferritin in preterm labor and 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, hemoglobin and ferritin measurements were performed for all participants.

Results. Hemoglobin 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 hemoglobin 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 hemoglobin 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 anemia and a lower ferritin level are at risk of preterm labour, while those with anemia and high level of ferritin are at risk of PPROM among pregnant women.

Key words: Preterm labour; anemia; ferritin; 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 anemia and is the most widespread nutritional disorder in the world [3,4]. Anemia had the potential risk of increasing preterm birth in addition to many other maternal diseases during and after pregnancy [5]. Since anemia is the most common diagnosis among pregnant women [6], it could be significantly associated with preterm labor as well as other comorbidities [7].

Preterm premature rupture of membranes (PPROM) is defined as spontaneous rupture of fetal 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 etiology of preterm labour including maternal age, parity, level of education, pregnancy interval, previous history of miscarriage or preterm labour, antepartum hemorrhage, maternal hypertension, recurrent urinary tract infection, & 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 hypothesized 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 anemia and ferritin levels on preterm delivery and PPROM.

Materials and Method

A case-control study was carried out in the Department of gynecology & 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 & agreement of the health authorities. Gestational age is calculated from the last menstrual period for those women with reliable menstrual cycles & confirmed by ultrasound assessment of fetal biometry that was done in gestational age less than 20 weeks. For those women with unreliable or do not know their last menstrual period we depend on ultrasound measurement for fetal biometry at a gestational age of fewer than 20 weeks. The participants were divided into 3 groups:

- Group A: 75 pregnant with preterm labour & 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 **maternal** such as (Gestational & pregestational diabetes mellitus, thyroid disease, preeclampsia, eclampsia, chronic hypertension& heart disease, antepartum hemorrhage, urinary tract infection, acute febrile illness, chorioamnionitis, & smokers). **Fetal** such as (fetal distress, intrauterine growth restriction, intrauterine fetal death, & fetal anomalies).

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

A General & abdominal examination was performed. Preterm Labour is determined by documented uterine contractions by echocardiography (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 & dilatation were assessed by sterile speculum examination.

Preterm Prelabour Rupture of Membrane is confirmed by direct visualization of pooling of amniotic fluid during sterile speculum examination. Ultrasound evaluations for fetal biometry, fetal 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. Hemoglobin 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 hematology autoanalyzer.

Ferritin measurement: The sample is collected in a plane tube and then left for 20-30 min at room temperature for clotting; then the serum is separated by centrifugation at 4000 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 differing 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 had above gravida three and part two.

The gestational age was the same for all participants as BMI which was also equal across three groups.

The hemoglobin 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 hemoglobin and ferritin among groups.

The gross correlation of hemoglobin across all participants with age, gestational age, BMI, and ferritin showed that only ferritin had a weak and significant positive correlation with hemoglobin, while other variables did not show a significant correlation (Table 2). In addition, ferritin showed only a significantly weak correlation with hemoglobin (Table 3).

Hemoglobin 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 & control groups (Figure 2).

ROC curve showed that comparing preterm with control cases, a hemoglobin 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 hemoglobin 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 (odd's ratio 3.2, 95% CI 1.8-5.4). And for PPROM (as the reference group) groups, both Hb

(odd's ratio 14.3, 95% CI 6-34.3) and the ferritin level (odd's ratio 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 anemia 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 anemia 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 anemia on preterm birth [13], as well as to other global studies [14,15].

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 anemia in early pregnancy could contribute to an increased risk of preterm while later anemia, or late exposure to anemia in pregnancy did not [3]. This may be true since we did not assess when anemia started and how it evaluated in pregnancy with anemia 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 hemoglobin 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 hemoglobin 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.

Conclusion

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

Implications of these findings for clinical practice and/or future research: hemoglobin level of ≥10 and a ferritin level of ≥32 could be proposed as a helpful marker that can predict preterm labour. While hemoglobin 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 anemia nor the correction status of anemia whether the participants receive a treatment for preidentified anemia or not. This could be important factors to be considered for future research.

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 interest.

Ethical approval: Ethical Committee of the University of Kirkuk - college of medicine

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 upon reasonable request to the corresponding author.

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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	0.99*
<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%)	0.39**
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%)	
4 – 5	26 (34.7%)	19 (25.3%)	27 (36%)	72 (32%)	0.28**
≥ 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 (mg/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/m^2)					
Mean ± SD	30.2 ± 5.2	29.7 ± 3.9	30.2 ± 5.7	30 ± 4.9	0.78*

* ANOVA test

**Chi-Square test

Table 2: Person's correlation for hemoglobin

		Age (Years)	Gestational Age (Weeks)	BMI (Kg/Cm2)	S.Ferritin (ng/ml)
Hb. (gm/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. (gm/dl)	Age (Years)	Gestational Age (Weeks)	BMI (Kg/Cm2)
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

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

		P value	Odd's ratio	95% C.I.	
				Lower	Upper
Preterm	Gestational Age	0.690	0.979	0.880	1.088
	Hb. (gm/dl)	0.0001	3.217	1.890	5.476
	S.Ferritin (ng/ml)	0.945	1.000	0.989	1.011
	BMI (Kg/Cm2)	0.269	0.960	0.893	1.032
PPROM	Gestational Age	0.015	0.839	0.729	0.966
	Hb. (gm/dl)	0.0001	14.383	6.031	34.303
	S.Ferritin (ng/ml)	0.0001	0.958	0.941	0.977
	BMI (Kg/Cm2)	0.285	0.946	0.856	1.047

Figure 1: Distribution of hemoglobin (A) and S. Ferritin (B) across groups. A: Distribution of hemoglobin across groups (P=0.0001). B: Distribution of S. Ferritin across groups (P=0.0001). Independent-sample Kruskal-Wallis Test.

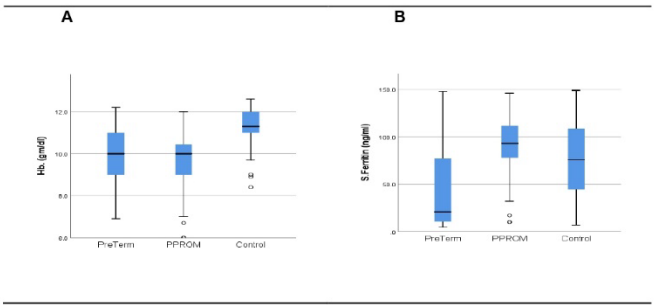

Figure 2: Distribution of hemoglobin (A) and ferritin (B) between groups according to age categories.

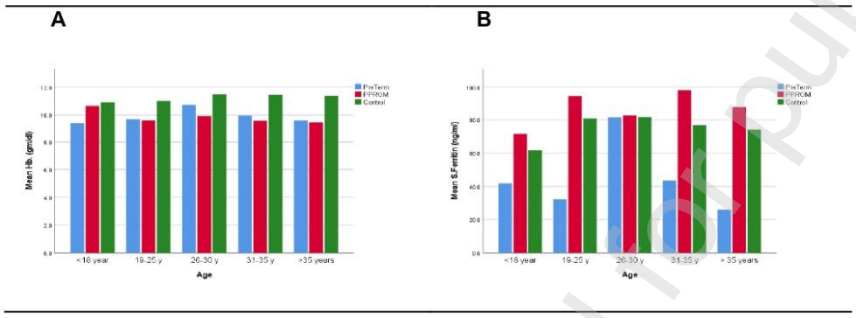

Figure 3: A receiver operating characteristic comparing preterm to control (B & D) and PPROM to control (A & C), respectively