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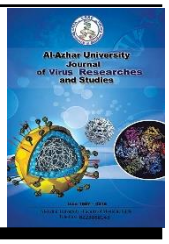

Serum Ferritin Levels in Pregnant Women with Preterm and Term Deliveries

Asmaa Hamed Mohammed[1], Hanaa Fathy Abo Ria[1] and Doaa Fathy Mohamed[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine for Girls, Al-Azhar University

***E-mail: motaweaasmaa@gmail.com**

Abstract

The measuring serum ferritin level as a sensitive inflammatory biomarker might effectively predict preterm delivery, but the power and the best cut-off point of this biomarker for predicting preterm labor in Egyptian population has not been substantially identified. This study aimed to evaluate serum ferritin levels in women with preterm and term delivery. This case control study was conducted on 90 gravid females who allocated into 2 groups: Group A: It comprised 60 cases with labor pain registered into 4 groups with a GA of 24 to 30 weeks, 30 to 34 weeks, 34 to 37 weeks, and >37 weeks (study group) and Group B: It included 30 cases who were in comparable GAs (control group) at Obstetrics and gynecology dep. at Al-Zahraa University hospitals. In this table, in preterm deliveries, ferritin levels were significantly higher ($P< 0.05$) in patients with PROM or a prolonged leakage; however, in term deliveries, there was no significant difference in ferritin level between patients with and without PROM or a prolonged leakage. In addition, ferritin levels were significantly higher in preterm deliveries than term deliveries in patients with PROM or a prolonged leakage ($p<0.001$). ROC-curve showed that the cutoff value of s-ferritin was 37.5 ng/ml with 79.5% and 84.3% sensitivity and specificity resp. for pre-and in-term delivery groups. The findings of the present study showed that serum ferritin level can be used to find patients at risk of preterm delivery.

Keywords: Ferritin, Pregnant, Term deliveries, Preterm.

1. Introduction

Preterm delivery, as the precedent of prematurity, is generally referred to childbirth before 37 weeks of pregnancy. It is considered not only as a main cause of neonatal mortality, but also, as a risk factor of behavioral problems even later through the child's life [1].

Recent experimental and clinical evidence have revealed an association between intrauterine inflammatory processes and preterm delivery. In fact, women with preterm delivery at less than 37 weeks have higher concentrations of inflammatory indicators in serum and amniotic fluid than those women who delivered at term [2]. In this context, a number of chemical and laboratory biomarkers have been studied for predicting preterm labor [2].

Ferritin has been identified as a diagnostic marker that its high serum levels is associated with a variety of acute phase reactions, including inflammatory

conditions. According to the main role of inflammation on appearance and progression of preterm delivery, it is hypothesized that the measuring 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 labor [3].

Preterm births continue to be the main cause of perinatal morbidity and mortality in developed countries, which increases the risk of neurocognitive and pulmonary deficits in surviving infants, The measuring serum ferritin level as a sensitive inflammatory biomarker might effectively predict preterm delivery, but the power and the best cut-off point of this biomarker for predicting preterm labor in Egyptian population has not been substantially identified. So, in this study; we aimed to compare of serum ferritin levels among cases with pre-term delivery and those with term delivery.

2. Patients and Methods

This was a case control study, which was performed at Obstetrics and gynecology department at Al-Zahraa University hospitals from March 2021 to November 2021. We enrolled 90 gravid females who allocated into 2 groups: Group A (case group with labor pain): It comprised 60 cases with labor pain registered into 4 groups with a GA of 24 to 30 weeks, 30 to 34 weeks, 34 to 37 weeks, and >37 weeks (study group) and Group B (normal pregnancy not in labor): It included 30 cases who were in comparable GAs (control group).

Exclusion Criteria

2.1 Inclusion Criteria

In this paper for study group: Single gestation and GA >24th week.

2.2 Exclusion Criteria

In this paper for groups: Anemia (hemoglobin levels <9.7 g/dL in the 2nd trimester and <9.5 g/dL in the 3rd trimester), high s-iron level (>178µg/dL in the 2nd trimester and >193µg/dL in the 3rd trimester), chronic infectious disorders, multi-pregnancy, embryonic irregularities, embryonic intra-uterine mortality, severe polyhydramnios, DM, alcohol usage, smoking and medications abuse and ferritin level garnishes counting rheumatoid arthritis, hemochromatosis, hyper-thyroids, adult-onset Still's disorder, leukemia, Hodgkin lymphoma, and multi blood transfusion. Women with obstetric problems such as multiple pregnancy, polyhydramnios, cervical incompetence or known uterine malformation, placenta previa, diabetes mellitus and preeclampsia were also excluded. Smokers and subjects with uncertain gestation were not recruited in the study.

2.3. Methods

Patients were subjected to: Verbal and written consent after they had been made aware of the purpose of the study.

2.4 Complete History Taking

Personal history including Name, Age, marital state, address**,** menstrual history: including age of Menarche, menstrual disturbance, dysmenorrhea, related symptoms, history Parity, present history: of chronic diseases and medication, past history of HTN, DM, family history of similar condition or diabetes, history of allergy to any medication and surgical history of operation, laparoscopic interference, treatment of hirsutism by Laser.

Gestational age was based on the last menstrual period and confirmed by ultrasound examination, prior to 20 weeks of gestation. Venous blood samples were drawn at 24 to 26 weeks of gestation and

analyzed for the serum ferritin level. Serum ferritin was assayed using the ferritin quantitative test system-sensitive for the normal ferritin range. All the pregnancies were followed until delivery. Women with spontaneous preterm delivery before 37 weeks (preterm delivery group, $n = 60$) and those who delivered at term (term delivery group, $n = 30$) were compared with respect to age and serum ferritin concentration.

2.5 Examination

General examinations: Vital signs (BP, Temp, HR, RR) and Signs of (Pallor, Cyanosis, Jaundice, and Lymph node enlargement). Abdominal and local clinical examination.

2.6 Obstetric Examination

Fetal lie, Fetal presentation and fetal engagements.

2.7 Laboratory Investigation

- CBC: Hb (%), RBCs, WBCs, platelet count.
- Blood groups.
- Coagulation profile (INR, APTT, platelets and fibrinogen).
- Liver function test: AST and ALT, s.albumin, s.bilirubin, s.gamma-glutamyl transferase (GGT), prothrombin period and INR.
- Renal function test: serum creatinine, blood urea and urine analysis.

2.8 Serum Ferritin Level

Blood samples were collected from the participants under sterile conditions and stored in iron-free tubes at room temperature. Serums of the samples were separated within two hours and stored at minus 20°C. Serum ferritin level measured via particle enhanced immunoturbidimetric method with a fully automatic analyzer. In addition, hemoglobin level was measured using a fully automatic spectrophotometer.

2.9 Particle Enhanced Immunoturbidimetric Method

Immuno-turbidimetry permits quantitative determinations of proteins by explicit antigen anti-body reaction.

2.10 Ethical Consideration

Study protocol had been submitted for approval by Institutional Review Board, Al-Azhar University.

2.11 Data management and Statistical Analysis

Collected data were coded, entered and analyzed by means of MS-Excel. Statistical analysis has been performed via SPSS-20.0 (IBM-USA). Descriptive statistics including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of serum ferritin in determining the presence or exclusion of the preterm labor were calculated. Receiver operator characteristic (ROC) curves were constructed to investigate the diagnostic power of the variable. The cut-off score was estimated for the prediction of preterm labor by the ROC curve analysis (the empirical point that maximizes sensitivity and specificity of the ferritin level for predicting of the preterm labor). P values of 0.05 or less were considered statistically significant.

3 .Results

Table.1 shows the incidence of nulli-parity in pre-term birth was significantly elevated than that in the in-term birth and controls (P value= 0.005). Furthermore, the incidence of some comorbidities like PROM, vaginal hemorrhage in early gestation, over-weight and obese, pre-term birth history in the cases and their relatives of 1st-degree. In Table.2 hemoglobin levels were

comparable among the study groups with statistical insignificant differences, while s-ferritin levels were significantly elevated between pre-term groups than normal groups of the same GA (p value<0.05).

Table (1): Comparison of gestation status and risk-factors of pre-term birth amid the studied groups.

	Pre-term Labor N=30	Term Labor N=30	Control group (24-37weeks) (n=30)	P value
Age <18 years or >40 years	4(13.3%)	2(6.7%)	3(10%)	0.902
Less than 18 months between pregnancies	23(76.7%)	4(13.3%)	0(0%)	**<0.001***
Pregnancy status ➢ **Nulliparous** ➢ **Primiparous** ➢ **Multiparous**	8(26.8%) 7(23.2%) 15(50%)	6(20%) 4(13.3%) 20(66.7%)	3(10%) 3(10%) 24(80%)	**0.005***
Overweight and obesity	24(80%)	20(66.7%)	10(30%)	**0.008***
Early pregnancy vaginal bleeding	18(60%)	4(13.3%)	5(16.7%)	**<0.001***
History of pre-term labor	18(60%)	2(6.7%)	1(3.3%)	**0.007***
History of pre-term labor in first-degree relatives	10(33.3%)	4(13.3%)	2(6.7%)	**0.030***
PROM	21(70%)	3(10%)	0(0%)	**<0.001***
Prolonged leakage (longer than 12 hours)	15(50%)	3(10%)	0(0%)	**<0.030***
Polyhydramnios	4(13.3%)	2(6.7%)	0(0%)	0.105
Congenital fetal malformations	2(6.7%)	1(3.3%)	0(0%)	0.185
IUGR	8(26.7%)	1(3.3%)	0(0%)	**0.016***
Uterine malformations	6(20%)	0(0%)	0(0%)	**0.001***
Anemia	4(13.3%)	2(6.7%)	0(0%)	0.617

Qualitative data represented as number and percentage. PROM: Pre-term ruptures of membranes, IUGR: intra-uterine growing retardation, Hb: hemoglobin. Comparing between pre- groups and in-term, chi square testing or fisher exact testing.

Table (2): Mean hemoglobin and s-ferritin among the study groups.

Variables	Group A				Group B			P values		
	Pre-term (24-30 wk.)	Pre-term (30-34 wk.)	Pre-term (34-37 wk.)	Term (>37 wk.)	Pregnancy 24-30 wk.	Pregnancy 30-34 wk.	Pregnancy 34-37 wk.	b	c	d
Hb(g/dl)	10.68 ± 0.8	10.22 ± 1.2	10.1 ± 1.3	10.40 ± 0.9	10.01± 0 .9	10.35± 0.7	10.33 ± 1.0	0.709	0.412	0.117
P value a	0.789				0.716					
Ferritin (ng/mL)	55.27 ± 13.6	57.77 ± 17.5	65.33 ± 11.1	35.13 ± 9.2	33.13 ± 3.5	33.10 ± 2.4	33.4 ± 4.3	**0.001***	**<0.001***	**<0.001***
P value a	**<0.001***				0.524					

Table (3): Comparing of ferritin levels in pre-and in-term births according to membranes status.

			Ferritin level (ng/mL)	P value
Pre-term labor (n =30)	PROM	No (n = 3)	56.3 ± 3.33	
		Yes (n=21)	67.47 ± 7.01	**<0.001***
	Prolonged leakage (longer than 12 hours)	No (n = 15)	56.89 ± 4.54	
		Yes (n=15)	70.55 ± 3.24	**<0.001***
Term labor (n = 30)	PROM	No (n = 27)	35.52 ± 2.89	
		Yes (n=3)	36.17 ± 2.22	0.452
	Prolonged leakage (longer than 12 hours)	No (n = 27)	33.45 ± 2.28	
		Yes (n=3)	36.85 ± 1.98	0.132
PROM (n = 24)		Pre-term (n = 21)	67.47 ± 7.02	**<0.001***
		Term (n = 3)	35.53 ± 2.33	
Prolonged leakage (longer than 12 hours) (n = 18)		Pre-term (n = 15)	70.55 ± 1.28	**<0.001***
		Term (n = 3)	30.75 ± 3.24	

Comparing between pre- groups and in-term, Man Whitney test.

In Table. 4, in pre-term births, ferritin level was significantly elevated (P value< 0.05) in cases with PROM or an extended leakage; but, in term births, there was nonsignificant change in ferritin levels among cases with and with no PROM or an extended leakage. Furthermore, ferritin level was significantly elevated in pre-term births than in term births in cases with PROM or an extended leakage (p value<0.001). In Table. 5 ROC-curve showed that the cutoff value of s-ferritin was 37.5 ng/ml with 79.5% and 84.3% sensitivity and specificity resp. for pre-and in-term delivery groups. Furthermore, the best cutoff level s.ferritin for pre-term birth in cases with PROM and an extended leakages was 44.5 ng/mL and 45 ng/mL, resp.

Table (4): Comparing of ferritin levels in pre-and in-term births according to membranes status.

			Ferritin level (ng/mL)	P value
Pre-term labor (n =30)	PROM	No (n = 3)	56.3 ± 3.33	
		Yes (n=21)	67.47 ± 7.01	**<0.001***
	Prolonged leakage (longer than 12 hours)	No (n = 15)	56.89 ± 4.54	
		Yes (n=15)	70.55 ± 3.24	**<0.001***
Term labor (n = 30)	PROM	No (n = 27)	35.52 ± 2.89	
		Yes (n=3)	36.17 ± 2.22	0.452
	Prolonged leakage (longer than 12 hours)	No (n = 27)	33.45 ± 2.28	
		Yes (n=3)	36.85 ± 1.98	0.132
PROM (n = 24)		Pre-term (n = 21)	67.47 ± 7.02	**<0.001***
		Term (n = 3)	35.53 ± 2.33	
Prolonged leakage (longer than 12 hours) (n = 18)		Pre-term (n = 15)	70.55 ± 1.28	**<0.001***
		Term (n = 3)	30.75 ± 3.24	

Table (5): Middle cerebral artery RI of the two studied groups.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Pre-term vs. term deliveries	>37.5	0.828	79.5%	84.3%	83.5%	81.1%	81.9%
PROM: Pre-term vs. term deliveries	>44.5	0.658	71.6%	64.6%	73.4%	69.3%	71.6%
Prolonged leakage: Pre-term vs. term deliveries	>45	0.672	77.5%	85%	83.8%	79.1%	81.3%

4. Discussion

Respiratory Ferritin is an iron storage protein. It is an acute phase reactant. Ferritin level increases in inflammation and infection. Some researchers have demonstrated that increased s-ferritin level is associated with pre-term labor [4]. This study aimed to perform a comparison of s-ferritin levels among cases with pre-term delivery and those with term delivery and as well gravid cases in 24-37th wks. of GA not in labor. In the present study, the s-ferritin levels were significantly elevated among pre-term groups than normal groups of the same GA (p value<0.05). But a no change was found between the groups of pre-term. Several studies have examined the relationship of s-ferritin with premature birth. In the research by Movahedi et al., [5] 222 single-ton pregnancies were referred to in Isfahan as University Hospital clinics. In terms of s-ferritin concentration, pre-term birth group (n:69) and birth group (n:153) have been compared. The mean concentration of the women pre-term delivered is greater than that of the pre-term ferritine (26.70±5.50 ng/ml) versus (19.80±3.60 ng/ml) p < 0.001.

This was in agreement with Salih, [6] as showed that the s-ferritin level significantly elevated in pre-term labor from controls and significantly elevated in PPROMs than controls. These findings were demonstrated graphically for s.iron and s-ferritin levels.

To add to our results, the mean levels of s-ferritin in pre-term was 76.30 ± 29.40 while in term was 20.20±5.0, so a significant change was found among the studied groups.**7** This was similar to other publications [8].

The probable clarification for elevated s-ferritin level in cases with pre-term labor and PPROM in this context can be iron excess or dormant chorioamnionitis, and as iron excess was omitted in the work by considering different hematological factors, consequently the elevated s-ferritin obtained in the pre-term and PPROM is more commonly a share of acute phase reactions to sub clinical infections [9].

In contrast to our findings, Valappil et al., [10] enrolled 50 pregnant women with PROM and 50 with pre-term labor. They found nonsignificant change in the ferritin values amid the controls and impulsive pre-term labor cases (p =0.18). They carried out their study on pre-term labor women during labor that may be the cause of difference.

In our results, among pre-term births, ferritin level was significantly elevated (P value< 0.05) in cases with PROM or an extended leakage; but, in term births, there was nonsignificant change in ferritin levels among cases with and with no PROM or an extended leakage. Furthermore, ferritin level was significantly elevated in pre-term births than in term births in cases with PROM or an extended leakage (p value<0.001).

In addition to our results, Jahedbozorgan et al., [11] demonstrated that in pre-term births, ferritin level was significantly elevated in patients with PROM or with an extended leakages (>12 h). Furthermore, in cases with PROM or extended leakages, ferritin level was significantly elevated in pre-term than in term births.

This goes in line with a study by Omar et al., [12] which conducted to compare between three groups. Group I: 70 women with spontaneous pre-term labor, group II: 70 women presented with PROM not in labor and group III: 70 women at the same gestational age with no risk of spontaneous pre-term labor with intact membrane as controls. In the current work, the s-ferritin level was high among pre-term labor and pre-term PROM when in comparison with control group with statistically significant differences. ROC curve in the present study showed that the cutoff value of s-ferritin was 37.5 ng/ml with 79.5% and 84.3% sensitivity and specificity resp. for pre-and in-term delivery groups. Furthermore, the best cutoff of ferritin levels for pre-term birth in PROMs and an extended leakages was 44.5 ng/mL and 45 ng/mL, resp.

Also, Jahedbozorgan et al., [11] found that ferritin levels of 37.5 ng/mL known as the optimum cutoff for pre-term birth, in comparison to the in term birth, and its sense, spec., and accuracy were 78.70%, 68.70%, and 73.60%, resp.

When comparing our results regarding roc curve for s-ferritin, Abdel-Malek et al., [7] revealed the cutoff point of s-ferritin amid the both groups was 31 ng/ml with sense 92.80%, spec. 99.40%, PPV 97.50%, NPV 98.40% and accurateness 98.30%.

Our findings weren't coincides with Elnasr and Ammar, [13] who demonstrated that the AUC for s-ferritin is 0.97, the cutoff value 110.5 ng/ml with sensitivity 86.7%, Specificity 94.1% and 95% CI 0.94-1.0.

Also, El-Shahawy et al., [14] conducted (ROC) curves which were constructed for s-ferritin as predictor of pre-term labor. S-ferritin was a highly significant predictor of pre-term delivery and more than 55 ng/mL was the optimal cut-off point.

Upon comparison hemoglobin level among our groups, our results revealed that hemoglobin levels were comparable among the study groups with statistical insignificant differences (p> 0.05).

Our findings was in agreement with Abdel-Malek et al.,[7] as they revealed that there was nonsignificant change as regard to Hb amid pre-and in-term groups.

Our results coincides with Omar et al., [12] who showed that the hemoglobin level was nearly comparable with non-statistical significant difference between pre-term groups and controls.

In contrast to our results, Khambalia et al., [15] reported that there is some indication of a U-shaped association among HB level in early gestation and the risk of pre-term birth.

In Singaporean study, investigation of 3728 births have been performed. It was noticed that females with anemia have elevated frequency/risk of pre-term birth at the time of birth than non-anemia. No more change in newborn outcomes were detected [16].

In our study, we found that the incidence of nulli-parity in pre-term birth was significantly elevated as compared to in term birth and controls (P value= 0.005).

This is goes in line with Elnasr and Ammar, [13] who revealed that there was high statistical difference was detected as regarding parity as the P value is 0.001 between term group and pre-term group.

In the present study, age and Hb levels have non-significant differences amid the pre- and in term groups, and amid each pre-term group and the controls with the same GA (P value> 0.05).

Similarly, Abdel-Malek et al., [7] demonstrated that a non-significant change among pre- and in-term groups as regard the demographics.

Our results were in agreement with the study performed by Elnasr and Ammar,[13] among 100 pregnant women between 20 to 24 weeks of gestation, 85 of whom were born at full term (37:39 weeks). Of these, 15 were premature (33-36 weeks) and non-significant changes were found as regard maternal age between term and premature group as the mean age for the term was 28.11 and 26.93 for the premature group was 0.35.

Although there is not enough evidence from well-controlled experimental studies to advocate routine use such prediction scale in improving maternal or neonatal outcomes, future advance in developing combined biochemical-sonographic scales to provide a very high sensitivity and specificity may be promising to expect a definitive role for them in improving maternal or neonatal outcomes. Nevertheless, the results of this study indicate that high levels of serum ferritin in singleton pregnancies may alert the clinician of the risk of preterm delivery.

5. Limitations of the study

Our design was a case control study; therefore, we suggest conducting further multicenter prospective studies to evaluate the predictive value of serum ferritin levels in different high-risk groups and compare it with other biochemical parameters of preterm delivery such as fetal fibronectin.

In fact, our obtained cut point was notably lower that were reported in previous similar studies and thus might be accompanied by higher accuracy for discriminating preterm and term labor. However, it seems that a serial estimation of serum level rather than a single value may give a clue to the possibility of preterm delivery that should be considered in further studies.

6. Conclusion

Higher s-ferritin levels are highly significant correlated with spontaneous pre-term birth and s-ferritin values >37.5 ng/mL was the best cutoff level.

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