ORIGINAL ARTICLE

The diagnostic value of cervicovaginal and serum ferritin levels in midgestation time to predict spontaneous preterm delivery

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ABSTRACT

Background: The aim of this study was to determine diagnostic value of cervicovaginal ferretin and serum ferretin levels at midgestation time in predicting preterm delivery in singleton pregnancies. **Patients and Methods:** A diagnostic test study through a prospective cohort design was carried out on 300 singleton pregnant women in 2012. A blood sample was obtained from all the patients within 22-24 gestational weeks for laboratory assessment of serum ferretin, and cervicovaginal sample was also taken to assess cervicovaginal ferritin level. Ferritin levels were compared between term and preterm deliveries at 37, 34 and 32 weeks of gestation. Receiver operating characteristics (ROC) curves were plotted to assess the diagnostic test values. **Results:** Mean serum ferritin level was 55.38 [standard deviation (SD 23.8)] ng/mL in term deliveries versus a mean of 91.27 (SD 25.2) ng/mL in preterm deliveries, which showed a statistically significant difference ($P < 0.001$). The ferritin levels in cervicovaginal term delivery group had mean of 11.29 (SD 16.2) ng/mL compared with a mean of 21.95 (SD 10.1) ng/mL among those with preterm delivery before 37 weeks of gestational age($P < 0.001$). The cervicovaginal ferritin level had a moderate to good diagnostic value with an area under curve being above 0.8 for all assessments. The serum ferritin level had a moderate to good diagnostic value with an area under curve being above 0.8 for all assessments. In both tests, its diagnostic value was higher for predicting preterm delivery at earlier gestational age. **Conclusions:** The results of this study indicate that high levels of serum and cervicovaginal ferritin in singleton pregnancies may alert the clinician of the risk of preterm delivery. Serum and cervicovaginal ferritin measurement at midgestation may be used as a predictive scale for preterm delivery in singleton pregnancies.

Key words: Cervicovaginal ferritin, ferritin, prematurity, preterm labor

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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 behavioural problems even later through the child's life.[1,2] About 12.8 % of pregnancies in USA lead to preterm birth, 3.66% being under 34 weeks of gestation. It occurs in 6%-10% of deliveries in developed countries.[3]

In many countries, the proportion of babies who are born prematurely has risen in the past 20 years. The increased prevalence could be attributed to the change in incidence of twin or multiple pregnancies, improved prenatal care and increased detection of preterm labour due to wider use of ultrasound in estimating the gestational age.

Premature birth is the major cause of hospitalisation of the mother before 37 weeks of pregnancy.[4] On the contrary, it is responsible for 75%-80% of infant mortalities.[5]

The lower the gestational age at birth, the incidence and severity of complications is higher and has a worse prognosis. Respiratory distress syndrome rate is decreased after 33 and 34 weeks of gestational age but still involves

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about 6% of infants born between 35 and 38 weeks of pregnancy. The economical and psychological burden of problem should also be taken into account.[4]

Although the physiology and pathology of spontaneous preterm birth is not well-defined, there is some evidence for the role of upper genital tract infections through activation phospholipase and production of prostaglandins or other inflammatory agents.[6] Research is ongoing to find appropriate laboratory methods to predict preterm delivery in order to minimize its potential maternal and neonatal complications. Both serologic and cervicovaginal sample assays have been of interest in this regard. The possible role of ferretin during inflammation has already been demonstrated.[7] Ferritin is mainly generated in liver, spleen, bone, placenta as well released by infiltrating leukocytes reacting against infections.[8,9] Several studies have been conducted on women with preterm delivery showing that serum ferritin levels were significantly higher among preterm delivery cases compared with the term pregnancies in control group. Since the patients with an overload of iron from these studies had been excluded, the main explanation remains to be an acute phase response to subclinical infection.[10,11]

Several studies have been conducted to assess role of serum ferritin or cervicovaginal ferretin in predicting preterm birth to investigate an association between them.[10,12-17] However, studies assessing their diagnostic value, especially regarding cervicovaginal ferretin, are quite scarce in literature. The aim of this study was to determine diagnostic value of cervicovaginal ferritin and serum ferritin levels at midgestation time to predict preterm delivery in singleton pregnancies.

PATIENTS AND METHODS

A diagnostic test investigation through a prospective cohort design was carried out in 2012 in a prenatal and obstetric care center. The study population included singleton pregnancies within 22-24 weeks of gestational age referring to ***(blinded) women's health center at ***(blinded) University hospital in Urmia, North-West of ***(blinded).

A total of 300 patients were primarily enrolled based on the sample size calculation to estimate sensitivity and specificity as a proportion measure presumed to be 80% with 5% precision to reach 95% confidence level and considering 15% attrition rate due to exclusion criteria or possible lost to follow-up cases.

The exclusion criteria comprised severe anemia, hypertension, smoking, known thalassemia, congenital anomalies, previous history of cervical insufficiency, premature rupture of membranes, cervical dilation, bleeding at sampling, placenta praevia, multiple pregnancies, chronic renal failure, diabetes, known thrombophilia, eclampsia and pre-eclampsia.

Demographic and background characteristics of the patients including age, weight, height, parity, abortions and spontaneous preterm delivery were recorded. The gestational age was assessed according to reliable Last Menstrual Period (LMP) dates or early pregnancy ultrasonographic study. Deliveries were classified in three different modalities as follows:

1. Preterm delivery: Spontaneous pregnancy termination before 37 weeks of gestational age
2. Early preterm delivery: Spontaneous pregnancy termination before 34 weeks of gestational age
3. Very early preterm (VEP) delivery: Spontaneous pregnancy termination before 32 weeks of gestational age

A blood sample was obtained from all the patients for laboratory assessment of serum ferritin, hemoglobin and hematocrit. Goldvac venoject sampling tubes were used.

Prior to pelvic examination, a speculum was used and the tip of the cervical swab brush was placed into the external cervical os while being rotated through 360°. The sampling swab was then transferred into a 0.12 sampling tube containing 1 cc normal saline. It was then shaked for 1 min before removing the swab and sealing the tube. Samples were sent to the laboratory where each sample was stored at –20°C until the samples were tested using radioimmunoassay (Yavaran Teb Company) in Urmia central health center laboratory.

The patients underwent routine prenatal control at Kosar women's health center. Patient delivery dates were retrieved from hospital registries or contacting them using the contact details recorded in the questionnaire. The research team had no interventions in pre-natal care process.

In the case of deliveries with severe preeclampsia, pregnancy termination due to that and premature rupture of membranes the subjects were excluded from the study. Pregnancies with preterm contractions were monitored.

Data were analyzed using stata version 11 statistical software package. Mean and standard deviations were reported for normally distributed numeric measures. Kendall's tau correlation coefficient, independent samples *t*-test and chi-square tests were used to explore the associations among various variables. Receiver operating characteristics (ROC) curves were plotted to assess overall test sensitivity and specificity.

Ethics statement: The study protocol was approved by the regional committee of ethics in Urmia University of Medical Sciences. The approval was based on the Iranian codes of research ethics stressing that informed consent should be obtained from all participants. In case of children/minors,

or those with impaired judgement or legal restrictions, consent should be obtained from parents or legal guardians of the the participants. In present study, all study subjects were authorised to give consent and they provided written informed consent to participate in the study based on the procedure approved by the ethics committee.

RESULTS

According to the inclusion criteria, 300 pregnant women were enrolled in the study, 20 of them being later excluded due to non-spontaneous preterm labor. Finally, a total of 280 patients in the age range of 15-45 years with a mean age of 26.6 years were studied, 172 of whom (61.4 %) were nulliparous. A previous history of still birth was seen in 14 (5%) women and 34 (12.1%) of the participants had a history of miscarriage. The body mass index (BMI) was in the range of 16.9-41.1 kg/m^2 with a mean of 26.5 kg/m^2.

Serum hemoglobin level was in the range of 10.5-15.8 g/dL with a mean of level of 11.75 g/dL. Considering the term gestational age at delivery to be at least 37 weeks, 45 patients had preterm delivery, 15 of whom (5.3%) had early preterm delivery before 34 weeks of their pregnancy and 7 patients (2.5%) had VEP delivery with a gestational age below 32 weeks in pregnancy.

Normal vaginal deliveries were done in 186 (64.3%) women, while 94 women had caesarean section deliveries. The minimum serum ferritin level was 10.9 ng/mL, with a maximum of 180 and a mean of 61.28 ng/mL. Mean cervicovaginal ferritin was 13.02 ng/mL with a minimum and maximum of 0.2 and 106 ng/mL, respectively. Cervical ferritin levels correlated poorly with serum ferritin when assessed using Kendall's tau coefficient (Kendall's tau-a = 0.4152, $P < 0.001$).

Comparing the patients according to the gestational age as preterm (<37 weeks) and term deliveries, no statistically significant difference was observed between the two groups in terms of age ($P = 0.25$), history of neonatal mortality ($P = 1.00$), serum hemoglobin levels ($P = 0.4$) and low BMI ($P = 0.14$).

Mean serum ferritin level was 55.38 [standard deviation (SD 23.8)] ng/mL in term deliveries versus a mean of 91.27 (SD 25.2) ng/mL in preterm deliveries, which showed a statistically significant difference ($P < 0.001$).

The ferritin levels in cervicovaginal term delivery group had mean of 11.29 (SD 16.2) ng/mL compared with a mean of 21.95 (SD 10.1) ng/mL among those with preterm delivery before 37 weeks of gestational age($P < 0.001$).

Comparing the patients according to the gestational age as early preterm (<34 weeks) and term/near-term deliveries, no statistically significant difference was observed between the two groups in terms of age ($P = 0.84$), history of neonatal mortality ($P = 0.54$), serum hemoglobin levels ($P = 0.07$) and low BMI ($P = 0.1$).

Mean serum ferritin level was between 58.98 ± 25.7 in term or near-term deliveries versus a mean of 99.4 ± 28.7 in early preterm deliveries, which showed a statistically significant difference ($P < 0.001$).

The ferritin levels in cervicovaginal secretions in term/ near-term delivery group had mean of 12.38 ± 15.93 ng/mL compared with a mean of 24.18 ± 8.57 ng/mL among those with early preterm delivery before 34 weeks of gestational age($P < 0.001$).

Comparing the patients according to the gestational age as VEP delivery with a gestational age <32 weeks, and term or near-term deliveries, no statistically significant difference was observed between the two groups in terms of age ($P = 0.58$), history of neonatal mortality ($P = 1.00$), serum hemoglobin levels ($P = 0.07$) and low BMI ($P = 1.00$).

Mean serum ferritin level was between 59.92 ± 26.03 ng/mL in term or near-term deliveries versus a mean of 109.04 ± 38.23 ng/mL in VEP deliveries which showed a statistically significant difference ($P < 0.001$).

The ferritin levels in cervicovaginal secretions in term near-term delivery group had mean of 12.68 ± 15.85 compared with a mean of 26.02 ± 6.86 among those with VEP delivery before 32 weeks of gestational age($P = 0.02$).

As observed in above comparisons, mean serum or cervicovaginal ferritin levels changed reversely with the gestational age.

Among nulliparous women, mean serum ferritin level was 92.64 ± 25.13 in preterm (<37 weeks) versus 55.23 ± 23.04 for term deliveries ($P < 0.001$). Among nulliparous women mean cervicovaginal ferritin level was 22.12 ± 9.30 in preterm (<37 weeks) versus 11.2 ± 1.55for term deliveries ($P < 0.001$).

To assess the diagnostic value of cervicovaginal ferritin level to predict spontaneous preterm delivery, the ROC curve was plotted [Figure 1a]. The area under curve was 0.81 [$P < 0.001$, 95% confidence interval (CI): 0.74-0.89]. To assess the diagnostic value of cervicovaginal ferritin level for spontaneous early preterm delivery, the ROC curve was plotted [Figure 1b]. The area under curve was 0.83 ($P < 0.001$, 95% CI: 0.75-0.92). To assess the diagnostic value of cervicovaginal ferritin level for spontaneous early preterm delivery, the ROC curve was plotted [Figure 1c]. The area under curve was 0.87 ($P < 0.001$, 95% CI: 0.79-0.94). The sensitivity and specificity, for a cutoff point of 16.8 ng/mL for cervicovaginal ferritin to predict preterm delivery, was calculated to be 86.7% and 80.3%, respectively. Based on the same cutoff value, the sensitivity and specificity

of predicting early preterm labor were 93.3% and 73%, respectively. The sensitivity and specificity for predicting very preterm delivery (<32 weeks) were 100% and 71.2%, respectively.

Figure 2 shows ROC graphs plotted to assess diagnostic value of serum ferritin. To assess the diagnostic value of serum ferritin level to predict spontaneous preterm delivery [Figure 2a], the area under curve was 0.85 (P < 0.001, 95% CI: 0.76-0.91). To assess the diagnostic value of cervicovaginal ferritin level for spontaneous early preterm delivery [Figure 2b], the area under curve was 0.86 (P < 0.001, 95% CI: 0.79-0.93). To assess the diagnostic value of cervicovaginal ferritin level for spontaneous early preterm delivery [Figure 2c], the area under curve was 0.87 (P < 0.001, 95% CI: 0.76-0.99). The sensitivity and specificity, for a cutoff point of 63.9 ng/mL for serum ferritin to predict preterm delivery, was calculated to be 91.1% and 72.5%, respectively. Based on the same cutoff value, the sensitivity and specificity of predicting early

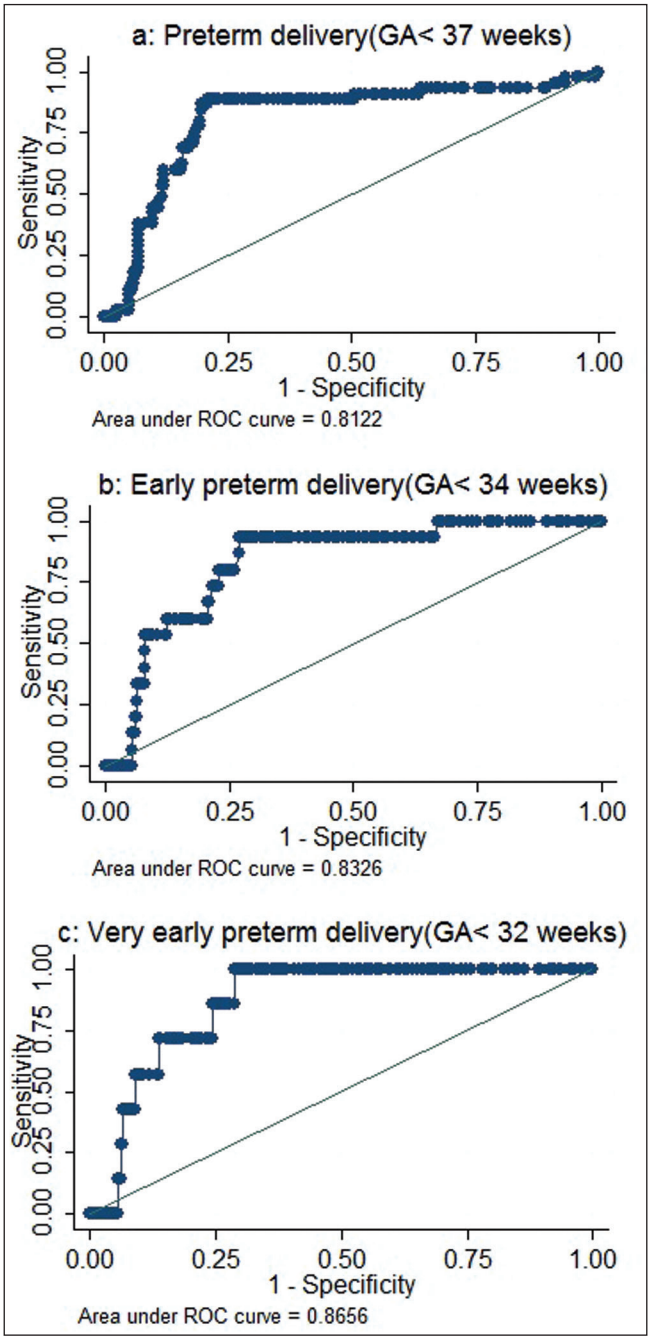

Figure 1: Receiver operating characteristics curves for diagnostic value of cervicovaginal ferritin at midpregnancy to predict spontaneous preterm delivery

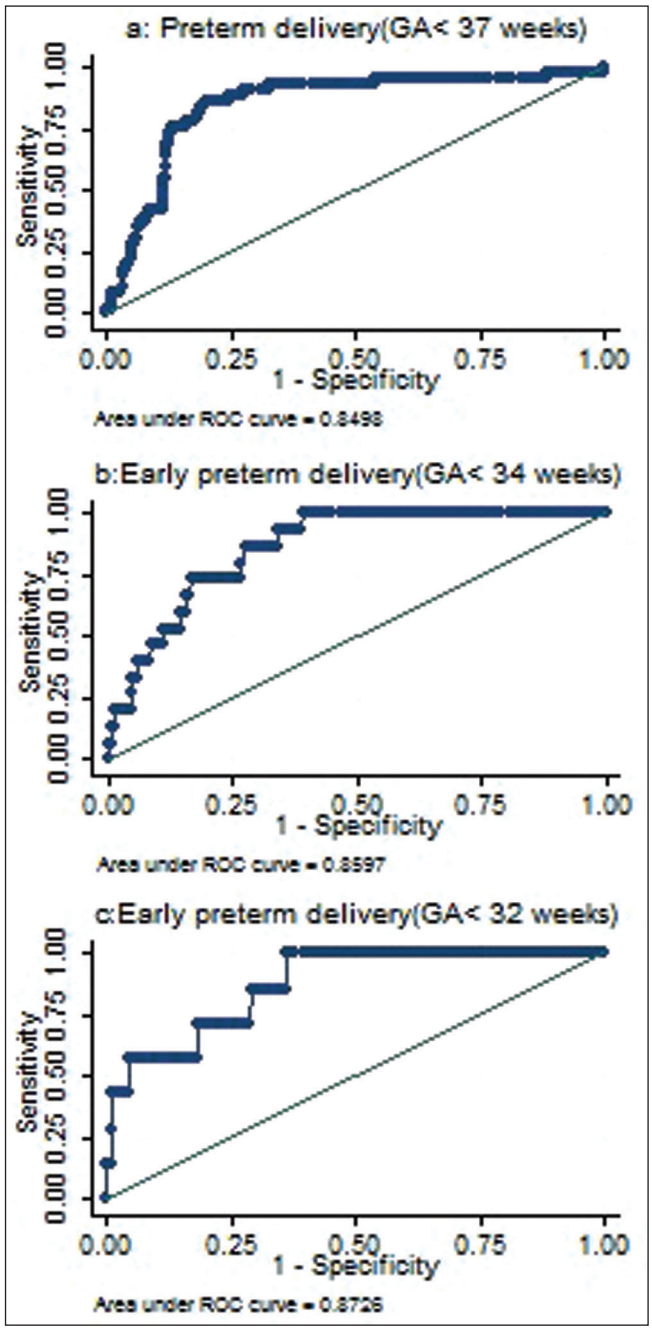

Figure 2: Receiver operating characteristics curves for diagnostic value of serum ferritin at midpregnancy to predict spontaneous preterm delivery

preterm labor were 93.3% and 65.4%, respectively. The sensitivity and specificity for predicting very preterm delivery (<32 weeks) were 100% and 63.8%, respectively.

DISCUSSION

This study investigated the role of cervicovaginal and serum ferritin levels in predicting preterm delivery. Serologic and cervicovaginal factors have been the focus of research and some studies have indicated that increased levels of inflammatory factors in pregnant women [interleukin (IL)6, granulocyte colony stimulating factor(G-CSF), ferritin] and the cervical and vaginal secretions of these agents (fetal fibronectin, lactoferrin and IL6) might be associated with preterm delivery.[2,3,17]

Ferritin plays an important role as host defense against bacterial invasion through sequestering iron, which is considered as a vital nutrient for bacterial growth. Placental isoferritin which is a placental isoform of ferritin has been shown to be expressed in syncytiotrophoblast, Hofbauer cells and decidual macrophages.[9,18] In present study, the midgestational serum ferritin level was higher for those who later ended in preterm delivery and even higher for those ending in a preterm delivery at lower gestational age. The serum ferritin level had a moderate to good diagnostic value. Several studies have investigated an association between serum ferritin and preterm delivery, some assessing a possible role for it in predicting preterm delivery.[9-16,19-21] Mostly, these studies have been indicative of an association between serum ferritin and preterm delivery.

With respect to the diagnostic value of serum ferritin in predicting preterm delivery, Very few studies have assessed sensitivity, specificity and the area under ROC curve for serum ferritin in predicting preterm delivery. Singh *et al*.,[15] in their study enrolled 40 women with preterm labor and 40 women who delivered at term. They compared several parameters including adrenocorticotropic hormone(ACTH), prolactin, thyroid-stimulating hormone (TSH), ferritin and alkaline phosphatase (ALP). Serum ACTH, ferritin and ALP were significantly higher in the subjects who delivered prematurely as compared with the controls. Ferritin was considered to be a better marker with area under curve equal to 0.96 as compared with 0.88 for ACTH. Descriptively, an area under curve of 0.96 seems to be of a high diagnostic value, but as the authors of the mentioned study have not provided the CIs, the precision of the provided value remains ambiguous. However, considering the small size of their study and through inspecting the ROC curves provided by them, one should be quite cautious in interpreting their results. Another study by Movahedi *et al*.,[12] has also examined the ROC curves for diagnostic value of serum ferritin. They followed 222 singleton pregnancies while recording serum ferritin levels at 24-26 weeks of gestation. Among these 222 women, 69 pregnancies ended in preterm deliveries. Mean serum ferritin was 26.7 ng/mL for preterm deliveries versus 19.8 ng/mL for term deliveries. A serum ferritin level of 22.5 ng/mL was stated to yield the best combination with sensitivity of 78.3%, specificity of 83.0%, positive predictive value of 67.5% and negative predictive value of 89.4% for prediction of preterm delivery. Unfortunately, they reported neither the CIs nor the area under ROC curve, which makes it difficult to appropriately interpreted the results. Various serum ferritin cutoff levels have been suggested in the literature to predict preterm delivery. In present study, a serum ferritin level cut point of 63.9 was selected providing a sensitivity equal to 91.1% and specificity of 72.2% in the diagnosis of preterm labor before 37 weeks. A sensitivity equal to 93.3% and specificity of 65.7% in the diagnosis of early preterm labour (<34 weeks) was estimated. In a study by Goel *et al*.,[13] serum ferritin levels greater than 40 ng/mL was selected as cutoff for the prediction of delivery before 34 weeks.

In present study, the midgestational cervicovaginal ferritin level was higher for those who later ended in preterm delivery and even higher for those ending in a preterm delivery at lower gestational age. The cervicovaginal ferritin level had a moderate to good diagnostic value. Its diagnostic value was higher for predicting VEP delivery. Despite a vast literature review, the evidence in this regard was quite scarce. Consistently with our results, Ramsey *et al*.,[9] found that the mean cervicovaginal ferritin level was higher for those pregnancies ending in a preterm delivery. The predictive values assessed in our study were found to consistently exist both for nulliparrus and multiparous pregnant women.

Cervicovaginal ferritin levels in present study correlated poorly with the serum ferritin levels. This observation, along with findings on their individual predictive value, raises the potential for concomitant use of them to predict preterm delivery. Although there is not enough evidence from well-controlled experimental studies to advocate routine use such prediction scales 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 and cervicovaginal ferritin in singleton pregnancies may alert the clinician of the risk of preterm delivery.

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