

## Study of serum high sensitive C-Reactive protein and ferritin in preterm labor

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### Abstract

**Introduction:** Preterm delivery is estimated to be one of the leading causes of perinatal morbidity and mortality, for which there are no known effective preventive measures. C - reactive protein (CRP) is one of the sensitive markers of systemic inflammation and ferritin is an acute phase reactant protein.

**Objectives:** To compare the serum levels of high sensitive C-reactive protein (hsCRP) and Ferritin between pregnant women presenting with preterm onset of labor followed by delivery and age matched women who delivers at term.

**Materials and Method:** The study included 50 women aged between 18-35 years with singleton pregnancy and gestational age between 24-34 complete weeks with the diagnosis of new onset of preterm labor and a control group of 50 healthy and age matched pregnant women in the term labor. In all the cases and controls, serum hsCRP and ferritin were measured by the latex enhanced immuno-turbidimetric methods using reagent kits from Agappe diagnostics.

**Results:** Serum mean levels of hsCRP and ferritin were significantly higher in preterm labour cases when compared to controls. The difference was statistically highly significant ( $p < 0.001$ ).

**Conclusion:** Present results demonstrated that the assessment of maternal concentrations of hsCRP and ferritin can be used as suitable biomarkers for predicting preterm labor.

**Keywords:** Preterm labor, hsCRP, Ferritin, Acute phase protein, Immuno-turbidimetric method.

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### Introduction

Preterm labor refers to the onset of uterine contractions of sufficient strength and frequency to effect progressive dilation and effacement of cervix between 20 and 37 weeks of gestation. Preterm labor accounted for 5-10% pregnancy complications.<sup>(1)</sup>

Preterm delivery is estimated to be one of the leading causes of perinatal morbidity and mortality in India. It accounts for nearly 70% of the neonatal mortality and 50% of long term neurological complications. Preterm labor remains a major management challenge particularly with regard to the respiratory and neurological outcome in infants.<sup>(2,3)</sup>

Clinical and much research experimental evidence indicate that preterm delivery results from four pathologic mechanisms, which include amniochorionic-decidual or systemic inflammation, activation of the foetal or maternal hypothalamic pituitary axis, pathologic distension of the myometrium and decidual hemorrhage.<sup>(4)</sup>

C - reactive protein (CRP) is one of the sensitive markers of systemic inflammation and is synthesized by liver in response to infection and tissue injury in body. Maternal serum concentrations of high sensitive C-reactive protein (hsCRP) can be studied as a marker of subclinical infection in pregnant women who end up with preterm labor or premature rupture of membrane.<sup>(5)</sup>

Anemia in pregnant women is one of the common medical problems in India and accounts for significant

morbidity and mortality. Low serum ferritin values are encountered in antenatal clinics, however ferritin being an acute phase reactant protein is increased in pregnant women predisposed to preterm delivery. Therefore evaluation of ferritin can be studied as a biomarker in preterm labor.<sup>(2)</sup>

Present study was undertaken to evaluate serum concentrations of hs-CRP and ferritin, in pregnant women with preterm onset of labor followed by delivery and age matched women who delivers at term.

### Objective

To compare the serum levels of high sensitive C-reactive protein (hsCRP) and ferritin between pregnant women presenting with preterm onset of labor followed by delivery and age matched women who delivers at term.

### Materials and Method

The study was carried out at BLDEU's Shri B M Patil Medical College, Hospital and Research Center, Vijayapur, Karnataka, India, during the period from October 2012 to March 2013. The study was approved by the Institutional Ethical Clearance committee. Subjects were enrolled in the study after informed consent. The participation of study subjects was kept confidential.

Cases included 50 women aged between 18-35 years with singleton pregnancy and gestational age between 24-34 complete weeks with the diagnosis of

new onset of preterm labor. Gestational age was based on the last menstrual period and confirmed by ultrasound examination. Preterm labor was defined as four contractions in 20 min, as eight contractions in 60 min or cervix dilation of more than 1 cm, with effacement of 80%. Control group included 50 healthy and age matched pregnant women in the term labor.

Subjects with conditions known to alter the serum hsCRP and ferritin levels such as chronic infective or inflammatory disorders, known malignancy, liver disease, upper respiratory tract infection, urinary tract infection, vaginitis, iron deficiency anemia, iron overload states, were excluded. Women with obstetric problems such as, multiple pregnancies, fetal anomalies, abruptio placenta, placenta praevia, pre-eclampsia, intrauterine growth retardation, diabetes mellitus, thyroid dysfunction and bad obstetric history were also excluded from the study. Smokers and subjects with uncertain gestation were not recruited in the study.

About 5ml of blood collected from large peripheral vein under aseptic conditions in plain vial for biochemical evaluation at admission to the hospital regardless of time of day. Serum was separated by centrifugation and stored at 4-8°C till use. Serum hsCRP and ferritin were measured by the Latex Enhanced Immuno-turbidimetric end point methods using reagent kits from Agappe diagnostics (Agappe Diagnostics Ltd., Ernakulam, Kerala, India) [The sensitivity of the test was 0.15 mg/L and 1 ng/mL and linearity limit was up to 10 mg/L and 750 ng/mL for hs CRP and ferritin respectively and Precision check was satisfactory for both the parameters].<sup>(6,7)</sup> Measurement of hsCRP is based on the principle that, CRP in the serum sample binds to specific anti-CRP antibodies, which had been adsorbed to latex particles and agglutinates. The agglutination is detected as an absorbance change. The magnitude of the change is proportional to the concentration of CRP in the sample.<sup>(6)</sup> The principle for the measurement of serum ferritin is, latex particles coated with anti-ferritin antibody (rabbit) are agglutinated when mixed with samples containing ferritin. The agglutination is directly proportional to the concentration of ferritin in the sample.<sup>(7)</sup>

**Statistical analysis:** Results are presented as mean  $\pm$  SD values. Unpaired 't' test is used for comparing different biochemical parameters between cases and controls. 'p' value of  $\leq 0.05$  was considered for statistical significance.

## Results and Discussion

Table 1 show that, serum mean levels of hsCRP and ferritin were significantly higher in preterm labour cases when compared to controls. The difference was statistically highly significant ( $p < 0.001$ ).

**Table 1: Comparison of serum mean levels of hsCRP and ferritin between cases and controls**

Groups	hsCRP (mg/L)	Ferritin ( $\mu$ g/L)
Controls (Mean $\pm$ SD)	2.15 $\pm$ 0.83	86.21 $\pm$ 7.47
Cases (Mean $\pm$ SD)	8.54 $\pm$ 1.56	172.37 $\pm$ 9.21
Controls vs Cases (Mean difference)	6.39	86.16
t*	25.56	51.28
p	< 0.001	< 0.001

\* Unpaired 't' test, p value < 0.001, highly significant

Preterm delivery is estimated to be one of the leading causes of perinatal morbidity and mortality in India for which there are no known effective prevention measures. C-reactive protein (CRP) is a sensitive inflammatory biomarker whose serum level increases during the infectious and inflammatory processes. CRP measurement is noninvasive and risk-free which can be used as diagnostic test for evaluating the risk levels and also anticipating the morbidity of both mother and fetus.<sup>(8)</sup>

In this study we discovered that high levels of maternal serum C-reactive protein were associated with increased risk of preterm delivery compared to women with normal CRP levels, those with elevated CRP levels more than 8 mg/L had a greater than two fold higher risk of preterm delivery. These findings suggest that elevated CRP levels an acute phase reactant protein associated with inflammation, could lead to the physiologic changes that result in preterm delivery.

Median concentration of serum hsCRP was higher in pregnant women with preterm onset of labor followed by delivery (8.54  $\pm$  1.56mg/L) compared to age matched women who delivers at term (2.15 $\pm$ 0.83mg/L). Our results are similar to a large, multicenter, prospective trial conducted by Sorokin and colleagues<sup>(9)</sup> who also found that elevated maternal serum concentrations of CRP were associated with preterm birth < 32 weeks of gestation.

C-reactive protein plays important role in the inflammatory process. CRP removes pathogens by binding to surface antigen and opsonizes them for uptake by phagocytes and also activate the classic complement pathway. CRP increases inflammation by induction of cytokines and tissue factor in monocytes. However, its main function is to reduce inflammation by decreasing migration of neutrophils to the site of inflammation, preventing neutrophils adhesion to the endothelial cells, and also helps in removal of nuclear antigens produced from necrotic and apoptotic cells. Other than inflammation, infection and tissue injury increased CRP levels associated with high body mass

index, smoking, hormonal therapy, and cardiovascular diseases.<sup>(10,11,12)</sup>

In the present study, we observed statistically significant elevation of mean serum ferritin level in patients with preterm labor ( $172.37 \pm 9.21 \mu\text{g/L}$ ) when compared to that of control subjects ( $86.21 \pm 7.47 \mu\text{g/L}$ ). This finding was in agreement with similar studies conducted earlier such as Ulmer and his colleagues,<sup>(13)</sup> who observed a correlation between an altered serum ferritin concentration and preterm labor and a cross-sectional study by Saha et al.<sup>(14)</sup> who also found significantly high levels of serum ferritin in patients who had preterm delivery. It seems that elevated mid-pregnancy serum ferritin levels can be predictive of spontaneous preterm delivery, especially those occurring at early gestational ages.

Ferritin, an iron storage protein, is an acute phase reactant. Ferritin is increased in inflammation and infection; some searchers have demonstrated that increased serum Ferritin level is associated with preterm labor. In our research we found that significant increase in Ferritin level in pregnant women presenting with preterm onset of labor followed by delivery compared to age matched women who delivers at term. Possible hypothesis for increased ferritin level in preterm labor in pregnancy increases the risk of vaginocervical infection due to altered vaginal pH. Even in asymptomatic subjects clinical or sub clinical infection can increase the risk of preterm labor or premature rupture of membranes.<sup>(2)</sup>

## Conclusion

According to the results of our trial we found that hsCRP and ferritin levels were high in pregnant women presenting with preterm onset of labor followed by delivery compared to age matched women who delivers at term. Assessment of maternal concentrations of hsCRP and ferritin can be used as suitable biomarkers for predicting preterm labor.

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