Serum Ceruloplasmin in Predicting Preterm Labour

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ABSTRACT

Introduction: Preterm birth, one of the unresolved problems in clinical obstetrics and one of the greatest threats to the developing fetus is the major cause of perinatal morbidity and mortality accounting for 85% of all early infant deaths. Increase in preterm delivery has been reported in last 25 years and thus the diagnosis of preterm deliveries continues to be a major goal in obstetric practice. The aim of this study was to explore the clinical utility of serum ceruloplasmin in predicting preterm labour.

Material and Methods: The study was designed to estimate serum ceruloplasmin by the kinetic method of Somani and Ambade based on ferroxidase activity and to follow them up to the term or otherwise to observe the outcome. Two samples were analysed, first sample in 22nd-26th week and second at 30th-34th week of gestation.

Results: In subjects having normal delivery and in subjects who developed preterm delivery, mean ± SD of serum ceruloplasmin at 22-26 weeks (2nd trimester) and 30-34 weeks (3rd trimester) was 1297 ± 294.79 IU/L and 1839 ± 247.517 IU/L and 1166± 234.1 IU/L and 1489 ±69.9 IU/L respectively. At 22 weeks cut off value of 1500 IU/L for ceruloplasmin, the sensitivity for preterm labour was 89% and the specificity was 61%, while positive and negative predictive values were respectively 15% and 94%.

Conclusion: With 142% rise in mean levels of serum ceruloplasmin in preterm subjects over normal subjects in 2nd trimester, high sensitivity, moderate specificity and high negative predictive values, serum ceruloplasmin ferroxidase activity at 22 weeks appears to be the economical, fast and a reliable potential screening tool in the diagnosis of preterm labour.

Keywords: Ceruloplasmin, Ferroxidase, Preterm delivery, Premature Rupture of Membranes.

INTRODUCTION

Preterm labour, defined as regular uterine contractions before 37 completed weeks of gestation with intact membranes with 4 cm or more cervical dilatation observable during a 2 hour period[1], leads to preterm birth which is defined by World Health Organization (WHO) as the delivery of an infant before 37 completed weeks (259 days).[2] Preterm birth is one of the unresolved problems in clinical obstetrics and one of the greatest threats to the developing fetus. According to WHO, 1 million children die annually due to complications of preterm birth out of the total 15 million preterm babies[3] and is the major cause of perinatal morbidity and mortality accounting for 85 % of all early infant deaths.[4] Despite tremendous advances in antenatal care, in contrast to decrease, increase in preterm delivery has been reported in last 25 years.[5] Cerebral palsy, impaired mental development, deafness, blindness, chronic lung disease, respiratory distress syndrome etc. are the major disabilities in preterm babies.[6] These morbidity adds not only to the economic cost but also has a great physical and psychological effect[7], and thus diagnosis of preterm deliveries continues to be a major goal in obstetric practice.

The exact etiology of preterm remains unclear, but inflammation has been implicated in the mechanisms responsible for preterm besides the other pathogenic processes. Ceruloplasmin, an acute phase serum protein has been reported to increase in cases of Premature rupture of membrane (PROM) and during inflammation.[8] Therefore this study was conducted to explore serum ceruloplasmin in predicting preterm labour.

MATERIAL AND METHODS

The study was designed to estimate serum ceruloplasmin and to follow them up to the term or otherwise to observe the outcome. Two samples were collected and analysed, first sample in 22nd - 26th week (second trimester) and second at 30th - 34th week (third trimester) of gestation.

The subjects were selected from the patients attending the antenatal OPD of Command Hospital (Southern Command). All the estimations were done in the Department of Biochemistry, AFMC, Pune. The personal, menstrual and obstetric history was recorded in proper proforma.

The study group included the women in second trimester attending the antenatal OPD in the age group of 18- 35 years. The exclusion criteria was elderly primigravida (above 35 years), previous history of Lower Segment Cesarean Section, subjects with diagnosed placenta previa, pregnancies with congenital malformations, unsure dates, any intercurrent acute or chronic illness. Informed consent was taken from all the subjects.

Fasting blood samples collected in sterile vials, were centrifuged at 2500 rpm for 10 minutes and serum was separated. Serum ceruloplasmin was estimated on the same day immediately by the kinetic method of Somani and Ambade.[9] This estimation is based on the principle that enzymatic oxidative property (that is ferroxidase property) oxidizes ferrous to ferric ion in acetate buffer of pH 5.4. The ferric ion then complexes with the chromogen. The formation of this ferric chromogen complex is measured kinetically at 376nm. The ferroxidase activity in serum was calculated from the factor and displayed directly by the fully automated analyser in IU/L.

Reagents and its preparation

i) Reagent-1: Chromogen (0.5 mmol/L) was made by dissolving 159.65 mg of norfloxacin in 1000 mL of acetate buffer (0.45 mol/L, pH 5.4) containing 0.2% Triton X-100.

ii) Reagent-2: Substrate (2.04 mmol/L) was made by

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sequentially dissolving, 320 mg of DTT and 800 mg of ferrous ammonium sulphate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, in 1000 mL of distilled water.

Both Reagent-1 and Reagent-2 were stable for more than 6 months at 4°C as well as at room temperature.

Assay Procedure:

Estimation was carried on fully automated analyzer analyzer XL 600 from Transasia Mannheim GmbH, Germany, which was set at following parameters: Assay Type: Rate A; Wavelength: Primary = 376nm, Secondary = 0nm; Slope of reaction: increasing; Assay Points 0, 0, 15, 18 cycles; Sample volume: 10µL; Reagent 1: 200µL; Reagent 2: 30µL; Technical limit: 1600IU/L; Factor 2010. For the blank correction, a blank was run using distilled water in place of the serum sample. The ferroxidase activity in serum was calculated from the factor 2010 fed into the analyser and displayed directly by the fully automated analyser in IU/L. *The method has been granted Govt of India Patent No192356.*

STATISTICAL ANALYSIS

Descriptive statistics like mean, percentages and SD were used to interpret the data. Microsoft office 2007 was used to make tables.

RESULTS

Of the 300 subjects in our study, outcome is available for 271 cases. Second sample of 121 patients could be collected. Total 21cases were found to develop PROM and preterm delivery.

Mean serum ceruloplasmin in subjects who had normal labour was found to decrease from 1297 ± 294.79 IU/L in the 2nd trimester to 1166 ± 234.1 IU/L in the 3rd trimester. Mean serum ceruloplasmin in subjects who developed preterm labour was found to decrease from 1839 ± 247.51 IU/L in the 2nd trimester to 1489 ±69.9 IU/L in the 3rd trimester as shown in table 1.

DISCUSSION

Serum and amniotic fluid C-reactive protein, serum ferritin[10], cervicovaginal fluid fetal fibronectin[11], cervical Phosphorylated form of insulin like growth factor binding protein-1(phIGFBP1), interlukin-6 in amniotic fluid, Matrix metalloproteinases-8,9 in cervical mucus plug[12], Pregnancy associated plasma protein A(PAPP-A)[13], Placental protein 13(PP-13), Salivary progesterone, salivary estriol, serum relaxin[14], enzymes like Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP)[10], Corticotropin releasing hormone, serum serpin B7[15], dual biomarker model i.e. albumin/vitamin D-binding protein[16], serum ceruloplasmin[17] etc have been reported to be associated with an increased incidence of preterm labor. Even the cervicovaginal secretion ceruloplasmin has been suggested by some workers as marker of preterm.[18] Serum ceruloplasmin increases in pregnancy as its synthesis is stimulated in the liver under the influence of estrogen.[19] Though we still could not identify the mechanisms that lead to preterm labour[20] but it is presently thought to occur due to an inflammatory pathology. An effective management of PROM can reduce the incidence of preterm labour and prematurity to a large extent. Some of the studies have shown variability in results across the studies. Therefore, levels of one of the least studied marker out of various markers, serum ceruloplasmin in the second and early third trimester of pregnancy was evaluated to find out its utility as marker of preterm labour.

Mean serum ceruloplasmin at 22 weeks of those subjects who eventually developed preterm labour was found to be higher (mean ± SD of 1839 ±247.51 IU/L) than those who did not develop preterm labour (mean ± SD of 1297±294.79 IU/L) and this increase was found to be highly significant ($p<0.0001$). It is in accordance to study conducted by Ogino et al[21] which showed ceruloplasmin in cervicovaginal secretions was significantly higher in PROM cases ($p<0.001$) than non PROM cases. The serum ceruloplasmin values at 22 weeks, only 3 cases of preterm was found in the second tertile whereas 38 cases with normal outcome were present in the upper tertile along with 18 cases of preterm labour. Serum ceruloplasmin cut off value of 1500 IU/l at 22 weeks had a sensitivity of 89% whereas the specificity was 61%. Serum ceruloplasmin had very high negative predictive values (94%) but low positive predictive values (15%). Thus a high serum ceruloplasmin predisposes a patient to preterm but a low value nearly rules out preterm labor. A positive correlation (r=0.1) was found between ceruloplasmin and preterm labour.

Mean serum ceruloplasmin at 30-34 weeks of those subjects who eventually developed preterm labour was found to be higher (mean ± SD of 1489 ± 69.6 IU/L) than those who did not develop preterm labour (mean ± SD of 1166± 234 IU/L). However this increase was found to be statistically not significant ($p=0.07$). A positive correlation was also found between ceruloplasmin in third trimester and preterm labour ($r=0.08$) though this correlation was not very strong. Also patients who developed preterm were divided into 2 groups on the basis of their period of gestation at the time of delivery. The group that delivered before 34 weeks had a lower ceruloplasmin (mean ± SD of 1825± 308 IU/L) than the group that delivered after 34 weeks (mean ± SD of 1856±188 IU/L). However this difference was not significant ($p=0.86$). This is in accordance with the study conducted by Vitoratos et al.[22] Erdinc et al[17] showed no statistical significance in maternal serum and amniotic fluid ceruloplasmin levels in preterm delivery. The difference in the result might be due to difference in methodology for ceruloplasmin estimation.

Ceruloplasmin is a major antioxidant in serum and its antioxidant action has been proposed as a crucial function of ceruloplasmin with highest oxidizing activity for Fe+2, thereby

	Normal Delivery		Preterm Delivery	
	First Sample (22-26 wks) (n=250)	**Second Sample (30-34 wks) (n=121)**	**First Sample (22-26 wks) (n=21)**	**Second Sample (30-34 wks) (n=21)**
Mean ±SD	1297±294.79 IU/L	1166±234.1 IU/L	1839±247.51 IU/L	1489±69.9 IU/L
Median	1364 IU/L	1233 IU/L	1639 IU/L	1482 IU/L
Range	650-1693 IU/L	844-1405 IU/L	1477-2293 IU/L	1437-1569 IU/L

Table-1: Ceruloplasmin levels in 1st and 2nd Sample in Normal Delivery and in Preterm Delivery

proposed ferroxidase as the alternative name. In this study, specifically the ferroxidase activity of ceruloplasmin was estimated. The increased ferroxidase activity in PROM might be the compensatory rise in antioxidant defence mechanism as ferroxidase activity is a measure of antioxidant activity.[23,24]

Collagen is a presumably essential for mechanical integrity and stress tolerance of amniotic membrane and thus the collagen damage caused by increased ROS formation leading to oxidative stress might lead to PROM. Increased conc of markers of oxidative stress has been reported in tissues from deliveries with PROM.[25-27] PROM is characterized by increased concentration of biomarkers of oxidative damage[28] and association between oxidative stress and PROM had been reported by Longini et al.[29] Ceruloplasmin being an acute phase reactant increases with inflammatory responses. The variation in the concentration of ferroxidase activity in different trimesters might be due to different inflammatory responses in the different trimesters of pregnancy.[30]

CONCLUSION

Majority of suggested biomarkers of PROM require techniques like Enzyme linked immunosorbant assay (ELISA), Radio immunoassay (RIA), High performance liquid chromatography (HPLC) etc. which are costly and time consuming. Of all the markers the estimation of serum ceruloplasmin ferroxidase activity is the most economical and can be estimated within a minute at a reagent cost of around one rupee. Thus, considering 142% rise in mean level in preterms subjects over normal subjects in 2nd trimester, high sensitivity, moderate specificity and high negative predictive values, serum ceruloplasmin ferroxidase activity at 22 weeks appears to be the economical, fast and reliable potential screening tool in the diagnosis of preterm labour.

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