Potential Biochemical Markers for Preterm Labor: A Pilot Study in North India

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Received: 15 December 2009 / Accepted: 24 September 2010 / Published online: 16 November 2010
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Abstract Preterm delivery is a major contributor for neonatal mortality. Intensive research is underway to establish a reliable biomarker that can ascertain the risk of preterm delivery in pregnant women. The aim of our study was to evaluate the role of various biochemical parameters as potential biomarker for risk assessment for preterm labor. Forty women presenting with preterm labor and 40 women who delivered at term were included in the study. Parameters that were evaluated include corticotrophin (ACTH), prolactin, thyroid stimulating hormone (TSH), ferritin and Alkaline Phosphatase (ALP). Serum ACTH, ferritin, ALP and Ferritin/Iron ratio were significantly higher in the subjects who delivered prematurely as compared to the controls. Comparison of sensitivity, specificity, likelihood ratio, positive and negative predictive values for different cut offs for ACTH, ferritin, ALP and ferritin/iron ratio was carried out. Ferritin emerged as the best marker with area under curve of 0.96 as compared to 0.88 for ACTH, 0.825 for ALP and 0.735 for ferritin/iron ratio. Our study establishes the superiority of ferritin as a predictive biomarker for preterm labor as compared to the rest of the parameters evaluated.

Keywords ACTH · Ferritin · Ferritin/iron ratio · Preterm labor

Introduction

Preterm delivery is one of the leading causes of perinatal morbidity and mortality. It accounts for nearly 70% of the neonatal deaths and 50% of long term neurological sequelae. Preterm labor 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 a 2 h period [1]. If preterm labor leads to an early delivery, the premature newborn is at risk for problems related to incomplete development of its organ systems.

Intensive research is underway to establish a reliable biomarker that can ascertain the risk of preterm delivery in pregnant women. Currently the accepted markers of onset of preterm labor are assessment of fetal fibronectin in cervicovaginal secretions and cervical dilatation; however fetal fibronectin levels are able to stratify females who won't deliver prematurely after the onset of symptoms effectively. Hence there is an urgent need to discover reliable indicators of premature labor.

According to the model studied by Lockwood et al., four pathogenic processes namely activation of maternal or fetal hypothalamic pituitary adrenal axis, decidual chorioamniotic systemic inflammation, decidual hemorrhage and pathological distension of the uterus precipitates premature delivery [2]. Several biologic and biochemical markers have been recently studied that may allow early identification of patients at risk of preterm delivery. Research is ongoing to identify a chemical or physical marker that predicts whether and when premature delivery will occur.

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With this basic knowledge we aimed to investigate whether serum ACTH, TSH, prolactin, ferritin, iron and ALP concentrations in women diagnosed with preterm labor can be of potential clinical value in the assessment of the risk of preterm birth.

Materials and Methods

Study was conducted in the department of Biochemistry, GB Pant hospital in collaboration with a private maternity clinic based in Delhi. Forty women presenting with preterm onset of labor followed by delivery (regular intra uterine contractions, dilatation or effacement of cervix, and/or ruptured membranes) between 28 and 34 weeks of gestation were enrolled in the study after informed consent. Forty chronological and gestational age matched women who delivered at term served as controls. Women with multiple pregnancies, fetal anomalies, abruptio placenta, placenta praevia, preeclampsia, intrauterine growth retardation, diabetes mellitus, thyroid dysfunction, bad obstetric history, anemia (Hb < 11 g %) and clinical signs of infection were excluded from the study.

Samples from the patients were obtained in a plain vial for biochemical evaluation, at admission to the hospital regardless of the time of day when patient was in labor and before the administration of any medications. ACTH, prolactin, TSH and ferritin were estimated by Roche Elecsys modular analytics Cobas e 411 using electrochemiluminescence immuno assay (Manheim Germany) using commercially available kits. Iron and ALP levels were assayed on OLYMPUS (AU 400, Japan) using commercially available kits.

Informed consent was obtained from all the study participants and the ethics committee of our tertiary care hospital approved the study.

Statistical Analysis

Baseline characteristics of the study participants were expressed in mean $\pm$ SD and percentage. Mann–Whitney *U* test was used to analyze differences in baseline characteristics between the study groups and the control group. The association between the various parameters in different groups was evaluated using Spearman correlation coefficient. *P*-value of <0.05 was considered statistically significant. Receiver operating characteristics (ROC) curves were plotted for different parameters to ascertain their discriminative value. Sensitivity, specificity, likelihood ratio, positive and negative predictive values were calculated to determine the best plausible biomarker among the parameters evaluated in the study. Statistical analysis was

performed using SPSS windows version 10.0 software (SPSS Inc., Chicago, Illinois).

Results

The mean age of the cases was 25.3 ± 6.6 years while that of controls was 26.3 ± 5.9 years. The mean levels of different parameters in study population are depicted in Table 1. Among the hormones the level of ACTH was significantly higher in cases as compared to control group (9.1 ± 6.3 vs. 2.5 ± 2.9 pmol/l, $P < 0.001$). The activity of ALP in cases was 276 ± 84.5 IU/l as compared to 184.2 ± 54.1 IU/l in the controls, ($P = 0.004$). A statistically significant difference was observed in cases and controls in ferritin and ferritin/iron ratio (169.9 ± 83.9 vs. 68.5 ± 24.2 μ g/dl, $P < 0.001$; 2.16 ± 0.88 vs. 1.35 ± 0.73 , $P = 0.03$, respectively). However no significant difference was observed in TSH, prolactin and iron between the two groups.

Figures 1, 2, 3, and 4 depict the area under curve for the parameters evaluated in our study. Ferritin emerged as the best marker with area under curve being 0.96 as compared to 0.88 for ACTH, 0.825 for ALP and 0.735 for ferritin/iron ratio. Comparison of sensitivity, specificity, likelihood ratio, positive and negative predictive values for different cut offs as determined by us for ACTH, ferritin, ALP and ferritin/iron ratio is depicted in Table 2.

Discussion

Intrauterine growth retardation and preterm delivery are the two main predisposing factors behind perinatal morbidity and mortality. A number of hormones, subclinical infection and decidual haemorrhage etc. have been implicated in its pathogenesis. A number of biomarkers have been studied to assess the timing of preterm labor. Our study aims to be a step further in this endeavor.

The circulating ACTH levels rise steadily during gestation. The cause(s) of this increase in ACTH may include

Table 1 Comparison of the various biochemical parameters among the cases and controls

Parameter	Cases	Controls	<i>P</i> value
ACTH (pmoles/l)	9.1 ± 6.3	2.5 ± 2.9	<0.001
TSH (μ IU/ml)	3.2 ± 1.8	2.2 ± 0.8	0.124
Prolactin (μ IU/l)	175.8 ± 103.2	165.9 ± 57.4	0.895
Iron (μ g/dl)	91.3 ± 57.7	67.1 ± 36.7	0.243
Ferritin (μ g/dl)	169.9 ± 83.9	68.5 ± 24.2	<0.001
ALP (IU/l)	276 ± 84.5	184.2 ± 54.1	0.004
Ferritin/iron	2.16 ± 0.88	1.35 ± 0.73	0.039

Fig. 1 ROC curve of ACTH. Area under curve: 0.88, P value: 0.001

Fig. 3 ROC curve of ferritin/iron ratio. Area under curve: 0.735, P value: 0.039

Fig. 2 ROC curve of ferritin. Area under curve: 0.96, P value: <0.001

Fig. 4 ROC curve of ALP. Area under curve: 0.825, P value: 0.004

placental synthesis and release of biologically active corticotrophin releasing hormone (CRH) and ACTH, pituitary desensitization to cortisol feedback, or enhanced pituitary responses to corticotrophin-releasing factors such as vasopressin and CRH [3]. The synergistic stimulation of the feto-placental unit and the pituitary adrenal axis of both fetus and mother play a pivotal role in initiation of

parturition [4, 5]. Our study is in accordance with Makrigiannakis et al. who demonstrated the role of serum ACTH levels as predictive marker for premature labor [6].

Ferritin, an iron storage protein, is an acute phase reactant. Several studies have demonstrated that serum ferritin level is significantly raised in pregnant women with preterm labor and premature rupture of membranes (PROM) [1, 7]. Our results also echo these findings. It has been suggested that ferritin is produced by the macrophages that infiltrate

Table 2 Comparison of the various attributes for the cut offs for ACTH, ferritin, ferritin/iron ratio and ALP

	ACTH AUC—0.88, <i>P</i> = 0.001		Ferritin AUC—0.96, <i>P</i> < 0.001		Ferritin/iron AUC—0.735, <i>P</i> = 0.039		ALP AUC—0.825, <i>P</i> = 0.004	
Cut off value	1.58	2.57	68.8	112	1.63	2.19	193	243
Sensitivity	95	85	95	80	80	60	85	60
Specificity	70	80	80	90	60	90	70	80
Positive likelihood ratio	3.17	4.25	4.75	8	2	6	2.83	3
Negative likelihood ratio	0.07	0.19	0.06	0.22	0.33	0.44	0.21	0.5
Positive predictive value	86.4	89.5	90.5	94.1	80	92.3	85	85.7
Negative predictive value	87.5	72.7	88.9	69.2	60	52.9	70	50

the chorion decidual interface following bacterial colonization. Pregnancy tends to predispose to vagino-cervical infection due to altered vaginal pH. Even in asymptomatic subjects clinical or sub clinical infection can lead to increased ferritin which thereby can help in assessing the possibility of preterm delivery [8].

Anemia is a common finding in pregnant women in India. Low serum ferritin values are encountered in ante natal clinics. However ferritin being an acute phase reactant is elevated in women predisposed to preterm delivery. We, therefore propose the evaluation of ferritin/iron as a biomarker in this field. This ratio will provide us with a consolidated assessment of preterm delivery in anemic patients. However further studies are required to validate its efficacy.

Serum ALP activity is increased in pregnancy due to presence of placental isoenzyme. A number of studies have been conducted to evaluate the association of ALP levels with pregnancy outcome. Brock et al. showed a significant correlation between increased ALP levels with subsequent delivery of low birth weight babies [9]. Goldenberg et al. demonstrated that high ALP levels are associated with three fold increased risk for preterm delivery [10]. Our findings are in accordance with the preterm prediction study which evaluated the role of ALP in spontaneous preterm delivery [11].

Prolactin levels were also evaluated as a potential marker for preterm labor. However no significant differences were noted in the prolactin levels between the cases and controls. Our finding is in accordance with a similar study conducted by Mazor et al. [12].

Hypothyroidism and autoimmune thyroid disease in euthyroid women are associated with preterm delivery. TSH levels were evaluated as potential biomarker. However, no significant difference was noted. Stagnaro-Green reported a role of thyroid hormonal status in precipitating preterm initiation of parturition [13].

Efforts have been made to try and identify biochemical markers that could predict preterm labor. The parameters that were significantly different between the two groups—ACTH, Ferritin, ALP, ferritin/iron were then further

evaluated to determine their efficacy in predicting preterm delivery. The area under curves as observed by ROC curves of ACTH, Ferritin, ALP, ferritin/iron were 0.88, 0.96, 0.825 and 0.735, respectively. This establishes the superiority of ferritin as a predictive biomarker for preterm labor as compared to the rest of the parameters.

The two possible cut offs derived from ROC curve for ferritin were 68.8 and 112 µg/dl. At a cut off value of 112 µg/dl, sensitivity was 80% and specificity was 90%. If the value is lowered to 68.8 µg/dl (close to the mean of the control group), sensitivity increased to 95% and specificity was lowered to 80%. Comparison of the positive predictive value (PPV) for these two cut offs yielded similar values (90.48 and 94.1%). However, negative predictive value (NPV) for 68.8 µg/dl was 88.89% as compared to 69.2% for 112 µg/dl, respectively. Taking into account all the four attributes into consideration, we suggest that the discriminative value for 68.8 µg/dl as cut off point for ferritin as a biomarker for preterm labor is superior to 112 µg/dl. A ferritin value >112 µg/dl amongst the subjects who delivered preterm baby, would entail twice the likelihood of delivering prematurely than those having a lower ferritin levels than 68.8 µg/dl in the same group. However Goel et al. suggested that serum ferritin at cut off value >40 µg/dl can point towards probability of preterm delivery [14].

The results of ferritin/iron ratio as marker for preterm labor were conflicting. The two cut offs chosen from ROC curve were 1.63 and 2.19, respectively. The cut off value of 1.63 yielded a positive predictive value of 80% and a negative predictive value of 60%. The corresponding values for 2.19 were 92.3 and 52.9%, respectively. Hence, it is not possible to choose a possible cut off value from our data as the higher NPV for 1.63 is compromised by the lower PPV for this cut off. Similarly, 2.19 as cut off have lower discriminating capability than 1.63 in ruling out preterm labor. Although the specificity for the cut off 2.19 is 90% and that of the lower cut off value is 60%, we prefer to designate 1.63 as a better cut off value for ruling out preterm labor since the lower cut off value is closer to the control group and has a higher NPV than 2.19.

Evaluation for ACTH was performed at two cut offs 1.58 and 2.57 pmol/l. The two cut offs demonstrated similar positive predictive values (86.36 vs. 89.47%). The sensitivity for the two values was 95 and 85% whereas the specificity was 70 and 80%, respectively.

Although the NPV was lowered from 87.5 to 72.7%, the ability to rule out pre term labor was more pronounced for 2.57 pmol/l as its specificity was higher than that for 1.58 pmol/l.

Similar pattern was observed for ALP activity. The two cut offs chosen (193 vs. 243 IU/l) had similar PPV (85 and 85.7%) but markedly different NPV at 70 and 50%, respectively. The sensitivity and specificity for 193 was 855 and 70% and that for 243 was 60 and 80%, respectively. Although the specificity is higher at 243 IU/l but NPV and sensitivity were higher at 193 by a greater proportion, hence we may consider 193 IU/l to be a better cut off indicator as it is closer to the control mean.

Ferritin/iron ratio should be explored as a novel biomarker for preterm labor as it takes into consideration the prevalence of anemia in pregnancy, which is a major confounding variable factor for interpreting ferritin results.

Our study had some limitations. The major draw back is the small sample size. Secondly, the samples were collected at the time of delivery. The definitive role of a biomarker can be ascertained only on evaluating their role in predicting the disease in high risk population. Hence a prospective study evaluating the discriminative role of these biomarkers in subjects presenting with threatened preterm labor is imperative to substantiate these findings. This will facilitate the initiation of therapeutic interventions for maternal and fetal wellbeing.

Serum ferritin emerged as promising biomarker of preterm delivery from our study. However, the role of other biomarkers such as corticotrophin, ALP and ferritin/iron ratio cannot be ignored. Hence, a conglomeration of various biologic markers together might be useful in assessing risk of preterm delivery.

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