

Study of Prolactin in Cervicovaginal Secretion in Women with Preterm Labor and Normal Pregnancy

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ABSTRACT

Objective: To evaluate the diagnostic value of cervicovaginal prolactin levels for predicting preterm delivery in women with preterm labor.

Materials and methods: The preterm labor group and normal pregnancy group consisted of 75 and 150 patients between 24 weeks and 36 weeks of gestation. The preterm group was further subdivided into two groups. Study group Ia: patients who came up with preterm labor and given tocolytic but had preterm delivery ($n = 50$). Study group Ib: patients who came up with preterm labor and given tocolytic and delivered full term ($n = 25$). A single cervicovaginal measurement was made in all the groups.

Results: Cervicovaginal prolactin was highest in the study group Ia (11 ± 9.31 ng/mL) as compared to the study group Ib (4.61 ± 6.2 ng/mL) and control group (2.51 ± 5.1 ng/mL), and the difference was statistically significant ($p = 0.0311$; $p = 0.0000$). The optimal cut-off value of prolactin was greater than 7 ng/mL in study group Ia with sensitivity level of 78%, at a specificity of 80% with positive and negative predictive values of 88.64% and 64.52%, respectively. The area under the receiver operating characteristic (ROC) curve was 79% which indicates fair diagnostic accuracy of cervicovaginal prolactin in predicting preterm labor.

Conclusion: Prolactin is a potential biochemical marker which can be used alone or complementary to other markers for predicting preterm labor.

Keywords: Cervicovaginal, Human chorionic gonadotropin, Preterm labor, Prolactin.

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INTRODUCTION

Preterm birth (<37 weeks gestation) continues to be one of the most significant obstetrical problems in the world contributing to 28% of nonanomalous perinatal mortality¹ and nearly half of all cases of congenital neurological disability.^{2,3} The burden of preterm birth is disproportionately distributed across the world with highest rates in Africa and Asia (31% and 54%, respectively).⁴ The incidence of preterm birth in India has been reported to be 14.5%.⁵

While our understanding of human labor and the causes of preterm labor have advanced over the past decades, the ability to accurately predict preterm labor has remained elusive. Apart from the history and clinical markers, a number of biochemical markers in various body fluids have been proposed for the prediction of preterm delivery. Plasma urocortin, fetal fibronectin, and phosphorylated insulin-like growth factor binding protein-1 and β -human chorionic gonadotropin (HCG) in cervicovaginal fluid have been shown to be a potential predictor in several studies.⁶⁻¹⁰ However, reported sensitivities, specificities, positive and negative predictive values have varied considerably in different studies and are far from optimal in same.^{10,11} Therefore, the search for the ideal marker is still on.

Prolactin is produced in the chorionic cytotrophoblast, decidua, amnion, and placental syncytiotrophoblast during pregnancy.¹² Prolactin reaches its highest concentration in amniotic fluid (7 μ g/mL) during the second trimester and its concentration remains elevated throughout pregnancy.^{13,14} Prolactin can be considered as a possible marker for preterm birth for two reasons. (1) It is actively secreted by the decidua throughout gestation and (2) high concentrations are observed in the amniotic fluid. Therefore, prolactin in the vagina or at the external cervical os may indicate decidual membrane separation or subclinical membrane injury or rupture and can serve as an effective predictor of preterm birth.^{13,15}

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Therefore, this study was planned to evaluate the diagnostic value of cervicovaginal prolactin levels for predicting preterm delivery in women with preterm labor.

MATERIALS AND METHODS

This was a prospective study of one-year duration conducted on 229 women between 24 weeks and 36 weeks of pregnancy with or without labor pains. The study was approved by the local ethics committee, and written consent was obtained after detailed information was given to every patient selected for the study.

Women with singleton gestation accurately dated by early second-trimester ultrasonographic examination with cervical dilatation <3 cm, intact amniotic membrane, and absence of

other maternal and fetal complications were included in the study. Woman with fetal congenital anomaly, placenta previa, vaginal bleeding, pregnancy-induced hypertension, fetal growth restriction, fetal distress, and multiple gestation were excluded from the study.

All enrolled women were subjected to detailed history, examination, and routine antenatal investigations. Cervicovaginal secretion sample for prolactin estimation was obtained during speculum examination from all patients before digital examination of patient and prior to administration of tocolytic therapy if needed. The single sample was taken at the time of enrollment of the patients. For the control group, mean gestational age being 31 ± 5.4 weeks. For the study group, sample was taken at the time when patients came with symptoms and signs of preterm labor (mean gestational age for study group Ia: 29.4 ± 2.2 weeks and for study group Ib: 30.6 ± 4.2 weeks. A cotton tipped swab was placed just into the endocervical canal and then into the posterior fornix of vagina, each for 30 seconds, in order to obtain cervicovaginal secretion samples for prolactin assay. The swab was then placed in a tube containing 1 mL of saline solution, and the tube was shaken for 1 minute before the swab was disposed off. The samples were centrifuged for 5 minutes at the rate of 1500 revolutions per minute. The supernatant was quantitatively tested for the presence of prolactin. The estimation of prolactin was done by Accu-Bind (ELISA MICROWELLS) kit manufactured by Monoclonal Inc., Lake Forest, CA. The Prolactin Accu Bind ELISA test system has a sensitivity of 0.05 ng. This is equivalent to sample containing 1.0 ng/mL prolactin (PRL) concentration.

The sample size was calculated using the following formula:

$$n = \frac{z^2 \times p \times (1-p)}{d^2}$$

where $p = 0.9$ (90% sensitivity), $z = 1.96$, $d = 0.5$ (precision).

A total of 229 patients were followed till delivery and were divided into the following groups. Study group: patient who came up with preterm labor, tocolytics given, and then patient followed till delivery ($n = 75$). The study group was further subdivided into two groups. Study group Ia: patient who came up with preterm labor pain, tocolytics given but had preterm delivery ($n = 50$). Study group Ib: patient who came up with preterm labor, tocolytics given, and patient delivered full term ($n = 25$). Control group: patient with normal pregnancy with no preterm labor pain ($n = 154$). They were followed till delivery. Of 154, 150 delivered at full term and 4 patients had preterm delivery. As their number was so less, they were excluded from the study. Therefore, the total number of patients analyzed was 225 (after excluding 4 patients in control group who delivered preterm).

The categorical data were described as n (%) whereas continuous variables as mean \pm SD. Two-sample t test and Mann-Whitney test were used in group comparisons. One-way ANOVA was used to test the difference among >2 groups in a case of normally distributed data. Data were analyzed using statistical software package, STATA 11.2.

The receiver operating characteristic (ROC) curve analysis was used to establish the cut-off values for cervicovaginal prolactin in the prediction of preterm delivery.

RESULTS

The demographic and obstetric characteristics of the women enrolled in the study are shown in Table 1. There was no statistically significant difference between study group and control group with regard to maternal age, parity, body mass index, socioeconomic status, and gestational age.

Table 2 showed prolactin values obtained from cervicovaginal secretions in women delivered term or preterm. In the study group, the mean values of cervicovaginal prolactin were higher (9.41 ± 9.0 ng/mL) as compared to the control group (2.15 ± 5.1 ng/mL), and this was statistically significant ($p = 0.0000$). The study group Ia had the highest mean value of prolactin (11.81 ± 9.31 ng/mL) as compared to the study group Ib (4.61 ± 6.2 ng/mL) and control group (2.15 ± 5.1 ng/mL), and the difference was statistically significant. The mean latency period from testing to delivery in the control group was 7.2 ± 1.3 weeks. The mean latency period from testing to delivery was significantly shorter in the study group Ia as compared to the study group Ib (3.9 ± 2.1 vs 6.6 ± 1.2 , respectively, $p < 0.001$; power = 100%).

The receiver operator characteristic curve analysis (Fig. 1) was used to establish optimal cut-off values of prolactin in the cervicovaginal secretion in order to predict preterm delivery. The optimal cut-off value of prolactin was greater than 7 ng/mL in study group Ia with the sensitivity level of 78% (64.04–88.47, 95% CI), at a specificity level of 80% (59.30–93.17, 95% CI) with positive and negative predictive values of 88.64% and 64.52%, respectively. The area under the ROC curve was 79% (69.12–88.88, 95% CI) which indicates fair diagnostic accuracy of cervicovaginal prolactin in predicting preterm labor. The optimal cut-off value of prolactin in patients who came up with preterm labor pain, tocolysis given, and delivered full term (study group Ib) was ≥ 2.5 ng/mL.

DISCUSSION

Preterm delivery continues to be a leading cause of perinatal morbidity and mortality throughout the world. Recent advances

Table 1: Demographic and obstetric characteristics of study groups

	Control group	Study group Ia	Study group Ib	Significance
Maternal age in year	26.02 ± 3.5	25.20 ± 2.2	25.10 ± 3.2	NS
Parity	1.72 ± 0.8	1.85 ± 1.1	1.84 ± 0.9	NS
BMI	23.59 ± 2.0 kg/m ²	22.28 ± 2.6 kg/m ²	24.17 ± 2.1 kg/m ²	NS
Gestational age at admission	31 ± 5.4	29.4 ± 2.2	30.6 ± 4.2	NS

Table 2: Cervicovaginal prolactin values in different groups

Group	No.	Mean	Median
Study group Ia	50	11.81 ± 9.3	9.34
Study group Ib	25	4.61 ± 6.2	2.00
Control	150	2.15 ± 5.1	0.90
Total	225	4.75 ± 7.5	1.10

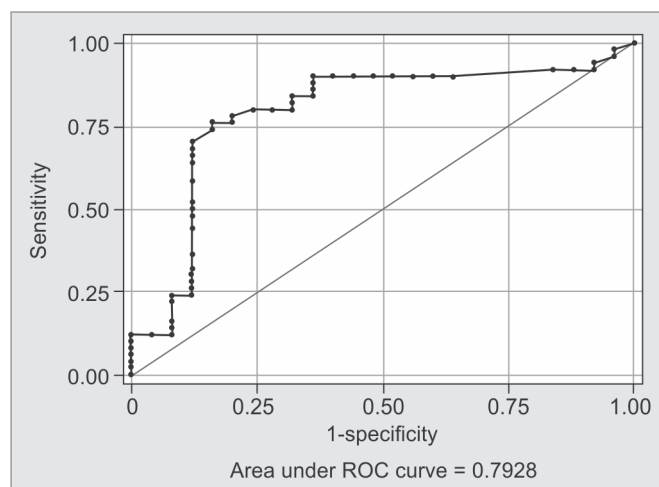


Fig. 1: ROC curve for prolactin for the prediction of preterm delivery

in perinatal healthcare have markers that facilitated more accurate prediction of preterm birth. The use of biological marker to enhance clinical approach in predicting preterm birth has been recently proposed. These tests have high potential to identify these symptomatic and asymptomatic patients who are at greater risk for preterm birth and who subsequently may require more intensive observation and interventions in terms of tocolysis and therapy for fetal lung maturation. Conversely, if low risk of preterm birth is diagnosed, the need for aggressive tocolysis, prolonged hospitalization, and close follow-up visits may be reduced. Till date, several biochemical markers have been studied but none have proved to be a gold standard.

In the present study, we have evaluated the diagnostic value of prolactin in the cervicovaginal secretion for predicting preterm delivery in women with preterm labor and in normal pregnancies. Prolactin was the first of many hormones recognized as a product of human decidualized endometrium.¹⁴ Prolactin is present in high concentration in amniotic fluid and may leak into the vagina following disruption of the decidua membrane interface which precedes onset of preterm labor.¹³ In our study, we observed that mean prolactin level in the women with preterm labor was three times higher than in control group (9.41 ± 9 mIU/mL vs 2.15 ± 51 mIU/mL), and the difference was statistically significant ($p = 0.000$). Our results were in good agreement with the study of O' Brien et al.¹⁵ and Guvenal et al.¹⁶

O' Brien et al.¹⁵ reported that prolactin was significantly identified in more women with preterm labor than in asymptomatic controls (50% vs 5%; $p < 0.0001$). Similarly, Guvenal et al.¹⁶ found significantly higher levels of prolactin in preterm group as compared to term delivery group (2.2 ± 17 mIU/mL vs 0.83 ± 0.66 mIU/mL, respectively, $p = 0.026$).

O' Brien et al.¹⁵ also observed that patients testing positive for prolactin had significantly shorter latency from testing to delivery (16 ± 17 vs 34 ± 24 days, $p = 0.02$) compared with patients testing negative. This was in concurrence to our study where patients with high prolactin levels delivered preterm despite tocolytic therapy.

The cut-off value of the cervicovaginal prolactin levels in prediction of preterm delivery in our study was greater than 7 ng/mL. The sensitivity, specificity, positive and negative predictive values of cervicovaginal prolactin for predicting preterm delivery were 78%, 80%, 88.64%, and 64.52%, respectively.

These values are high as compared to other studies (Table 3). O'Brien et al.¹⁵ gave the cut-off values greater than 2 ng/mL, positive predictive values of 80%, and negative predictive values of 65% in symptomatic patient with cervicovaginal secretion for delivery ≤ 34 weeks gestation. Prolactin values greater than 1.8 ng/mL when considered positive in the study of 17 women with preterm labor by Guvenal et al.¹⁶ According to these values, the sensitivity, specificity, positive and negative predictive values of the cervicovaginal prolactin for predicting preterm delivery were 50%, 96%, 67%, and 93%, respectively. Guvenal et al. also reported that cervicovaginal prolactin >1.8 ng/mL can identify 50% of women who will deliver preterm. Also, because of high negative predictive value of cervicovaginal prolactin for predicting preterm labor, unnecessary hospitalization and manipulative procedures can be avoided.

The other studies evaluated vaginal prolactin concentration in women with suspected but unconfirmed premature rupture of membrane. Buyukbayrak et al.¹⁷ and Kariman et al.¹⁸ gave the cut-off value of 30 μ IU/mL and 9.5 μ IU/mL, respectively, for vaginal washings prolactin in detecting premature rupture of membranes.

In the present study, we have observed that the cut-off values of prolactin in patients who came up with preterm labor pain, tocolysis given, and delivered at full term (study group Ib) was ≥ 2.5 ng/mL.

The difference in the sensitivity and specificity of various studies could be due to differences in the methodology used. Also, variation in the inclusion and exclusion criteria used and the characteristics of the patients studied (high or low risk for preterm delivery) can affect the cut-off value, sensitivity, and specificity of the cervicovaginal secretion prolactin for predicting preterm labor.

Also, we suggest that patients with preterm labor having a cut-off value of prolactin between 2.5 and 7 ng/mL are the high-risk group and need close monitoring and early intervention in the form of prophylactic tocolysis to prevent them from going into preterm labor.

Use of cervicovaginal prolactin as a biochemical marker has certain disadvantage. While the prolactin test appears to be informative in women with threatened preterm labor, it is generally poor positive predictive values^{15,16,18} and external factors (e.g., amniotic fluid contamination, vaginal bleeding, and unprotected sexual intercourse) that may limit its use as a lone predictor of preterm labor. Besides this, the method for collecting cervicovaginal

Table 3: Comparison of predictive values of cervicovaginal prolactin in published literatures

Studies	Cut-off value cervicovaginal prolactin (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
O'Brien et al., 1994 ¹⁵	>2	88	79	80	65
Guvenal et al., 2001 ¹⁶	1.8	50	96	67	93
Buyukbayrak et al., 2004 ¹⁷	30	95	78	93	84
Kariman et al., 2012 ¹⁸	9.5	87.03	75	75.80	86.53
Present study, 2018	>7	80	80	88.64	64.52

sample may also influence the results.⁹ Therefore, well-defined and standardized sampling methods for cervicovaginal prolactin may help avoiding misinterpretation of results and decrease the variations in the values due to technical differences in the experimental set-up.

On the other hand, cervicovaginal prolactin can be used clinically for its negative predictive value, which reaches 93% in some studies¹⁶ and will be particularly useful in determining whether a symptomatic patient in remote region requires emergency transfer to a tertiary healthcare facility.

Despite variations in the cut-off values of different studies, the significant point is that the raised cervicovaginal prolactin levels in women are at risk of preterm labor, and it can serve as a useful test for predicting preterm labor.

Our study has some limitations. The major drawback is the small sample size. Second, the samples were collected at the time when the patient had come with preterm labor. The definitive role of a biochemical marker can be ascertained only on evaluating their role in predicting the disease in high-risk population. Hence, a prospective study evaluating these biomarkers in early pregnancy and then following them for the development of preterm labor is imperative to substantiate these findings. This will facilitate the initiation of therapeutic interventions for maternal and fetal well-being in early pregnancy.

CONCLUSION

The data from this study suggest that prolactin is a potential biochemical marker which can be used for predicting preterm labor. Because of the multifaceted etiology of preterm birth, the desired predictive efficacy with single biochemical marker appears unlikely. Therefore, multiple biomarker modelling is suggested to predict preterm labor. The poor positive predictive value of cervicovaginal prolactin limits its role as a single marker to predict preterm labor. For this reason, we suggest that prolactin can be used simultaneously with other markers such as demographic/risk factors, cervical length, and other biochemical markers to improve diagnostic efficiency.

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