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Original Article

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Cervicovaginal Beta-hCG as a Diagnostic Biomarker for Prediction of Preterm Labor and its Co-relation with Cervical Length in Transvaginal Sonography

Dr. Riddhima Tripathi [1], Dr. Divya Dwivedi [2], Dr. Neena Gupta [3], Dr. Shaily Agarwal [3], Dr. Pavika Lal [2]

[1]Junior Resident, Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, India.
[2]Associate Professor, Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, India.
[3]Professor, Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, India.

*Corresponding Author: Dr. Riddhima Tripathi, *riddhimatripathi28@gmail.com*

Abstract

Background: To assess whether cervicovaginal β-human chorionic gonadotropin (β-HCG) and cervical length (CL) by transvaginal sonography predict spontaneous preterm delivery. ***Methods:*** In this prospective study, 150 singleton pregnancies at 24-34 weeks' gestation were enrolled at GSVM Medical College, Kanpur. 67 women who delivered before 37 weeks formed the preterm group, and 83 delivered at/after term formed term group. Cervicovaginal fluid was assayed for β-HCG (chemiluminescent immunoassay). Cervical length was measured transvaginally. Statistical analysis included t-tests, chi-square tests, ROC curves, and multivariate logistic regression for determining predictive value of beta-hcg and its co-relation with cervical length. ***Results:*** Mean cervicovaginal β-HCG was higher in the preterm group (27.8 ± 14.4 mIU/mL vs. 9.5 ± 9.9 mIU/mL; $p < 0.001$). Mean CL was shorter (2.58 ± 0.27 cm vs. 2.90 ± 0.32 cm; $p < 0.001$). Optimal β-HCG cutoff 10.2 mIU/mL yielded 88.1% sensitivity and 80.7% specificity (AUC= 0.845). CL cutoff ≤2.8 cm gave 82.1% sensitivity and 58.0% specificity (AUC = 0.774). Combined β-HCG and CL improved AUC to 0.936. On multivariate analysis, elevated β-HCG (adjusted OR 4.82, $p < 0.001$) and shortened CL (adjusted OR 0.38 per cm, $p < 0.001$) were independent predictors. ***Conclusion:*** Cervicovaginal β-HCG and TVS cervical length are effective, complementary predictors of preterm delivery. Their combined use enhances risk stratification and may guide early interventions.

Keywords: ***Cervicovaginal β-HCG, Cervical length, Transvaginal sonography, Preterm labor Predictive biomarkers***

Introduction

Preterm birth, defined as delivery before 37 completed weeks of gestation, complicates approximately 10-11% of pregnancies worldwide and is a leading cause of neonatal mortality and morbidity [1]. Early identification of women at risk of spontaneous preterm labor (PTL) is critical, as it allows for timely interventions such as antenatal corticosteroids, tocolysis, magnesium sulfate for neuroprotection, and neonatal intensive care preparedness, which significantly improve neonatal outcomes. However, predicting preterm delivery remains challenging due to the multifactorial nature of its etiologies and the limited accuracy of individual clinical indicators. Current approaches to PTL prediction rely on both ultrasound and biochemical tests. Transvaginal ultrasound measurement of cervical length is an established tool for assessing preterm birth risk. A short cervical length (typically ≤25 mm in mid-pregnancy) is a strong risk factor for spontaneous preterm birth, with large cohort studies demonstrating that the shorter the cervix, the higher the risk of early delivery [2]. For example, Iams et al. reported that women with a sonographic cervical length ≤25 mm at ~24 weeks have a substantially elevated risk of delivering before term, compared to those with longer cervices [3]. In symptomatic women presenting with threatened PTL, cervical length assessment can help distinguish true labor from false alarms, as a longer cervix makes imminent preterm birth unlikely. Another widely used test is the fetal fibronectin assay. Abnormal presence of Fetal fibronectin in cervicovaginal fluid between 22-34 weeks is a biochemical signal of membrane disruption. A positive fetal fibronectin test is associated with increased likelihood of preterm delivery, whereas a negative test has a high negative predictive value for ruling out imminent PTL [4]. Despite these advances, the positive predictive value of these tests is moderate at best, and a significant proportion of women with a short cervix or positive fibronectin do not deliver preterm. This has prompted investigation into additional biomarkers that might improve predictive performance. β-human chorionic gonadotropin (β-hCG) is a glycoprotein hormone produced predominantly by the placental trophoblasts. Small quantities of β-hCG have been detected in cervicovaginal secretions during pregnancy, and it has been hypothesized that an elevated level of β-hCG in vaginal fluid may reflect occult decidual hemorrhage or micro-separation of the chorion, processes that could presage the onset of labor. Notably, β-hCG measurement in vaginal fluid is already utilized clinically to diagnose premature rupture of membranes, where amniotic fluid leakage leads to a marked rise in vaginal β-hCG levels. In cases of

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prelabor rupture of membranes, vaginal fluid β-hCG concentrations are often dramatically higher (in the hundreds of mIU/mL) than in normal pregnancy, and a threshold of ~50 miu/mL has shown excellent sensitivity and specificity for membrane rupture [5]. By analogy, smaller increases in cervicovaginal β-hCG might occur in PTL even without gross membrane rupture, making β-hCG a plausible marker for impending preterm delivery. we designed the present study to reanalyze the diagnostic potential of cervicovaginal β-hCG for predicting spontaneous preterm labor and to clarify its relationship with the established sonographic marker of cervical length. By evaluating both markers concurrently in the same population, we aimed to determine whether β-hCG provides complementary predictive value to cervical length measurement. We also sought to identify optimal threshold values in our study and to assess the combined use of a biochemical (β-hCG) and biophysical (cervical length) test for improved prediction of preterm birth. Women with elevated cervicovaginal β-hCG levels would be more likely to deliver preterm, especially if they also had a short cervix, and that the combination of these factors would yield better predictive performance than either factor alone.

Materials and Methods

Study Design and Population

This prospective case-control study was conducted from March 2023 to March 2025 at GSVM Medical College, Kanpur, after Institutional Ethics Committee approval (Ref. EC/44/Feb./2024). Singleton pregnancies at 24-34 weeks' gestation were screened; 150 women consent completed follow-up. Inclusion criteria were women with history of preterm birth, pprom, with one or pregnancy losses, presenting with threatened miscarriage, willing to participate and follow up. Exclusion criteria included multiple gestation, structural uterine anomalies, prior cerclage, active labor at presentation, PPROM, iatrogenic preterm delivery, APH, aand major fetal anomalies.

Data Collection

At enrollment (24-34 weeks), demographic and obstetric history were recorded. Cervicovaginal fluid was collected via sterile swab at the external os and posterior fornix, eluted in saline, shaken for 1 min and sent to laboratory. β-HCG was measured by chemiluminescent immunoassay (mIU/mL). Immediately after cervical length was measured by TVS (transvaginal probe, minimal pressure) as the shortest of three sagittal measurements from internal to external oS. Routine antenatal care was followed till delivery. Female developing preterm labor were taken as study group and not developing preterm labor as control. Result was calculated using appropriate statistical tools.

Statistical Analysis

Continuous variables were compared with Student's t-test, categorical with chi-square. Correlation between β-HCG and CL used Pearson's r. ROC curves identified optimal cutoffs (Youden's index) and calculated AUC, sensitivity, and specificity. Multivariate logistic regression determined independent predictors. $p < 0.05$ denoted significance.

Observation and result

Table 1: Comparative Analysis of Demographic, Obstetric, Medical, Laboratory, and Neonatal Parameters Between Study and Control Groups

Variable	Study Group (n=67)	Control Group (n=83)	P-value	Statistical Method
Demographic Characteristics				
Age Group (years)			0.751	Chi-square test
22-24	17 (25.37%)	17 (20.48%)		
25-27	39 (58.21%)	50 (60.24%)		
28-30	11 (16.42%)	16 (19.28%)		
Socioeconomic Status			<0.001*	Chi-square test
- Lower (L)	33 (49.3%)	14 (16.9%)		
- Lower middle (LM)	20 (29.9%)	34 (41.0%)		
- Upper lower (UL)	14 (20.9%)	35 (42.2%)		
Obstetric History				
Gravida, n (%)			0.034*	Chi-square test
G1	6 (9.0)	20 (24.1)		
G2-G4	56 (83.6)	54 (65.1)		
>G4	5 (7.5)	9 (10.8)		
History of Abortion, n (%)			0.664	Chi-square test
Present	37 (55.2)	43 (51.8)		
Absent	30 (44.8)	40 (48.2)		
Past history of preterm, n (%)			<0.001*	Chi-square test
Yes	47 (70.1)	20 (24.1)		
No	20 (29.9)	63 (75.9)		
Current Pregnancy				
Gestational age (Weeks) at which β-HCG sample is taken, n (%)				
24-26 weeks	15(22)	10(12)	< 0.01*	Chi-square test
27-30 weeks	23(34)	31(37)	< 0.01*	
31-34 weeks	5(7)	1(1)	0.187	
Medical Comorbidities				
Hypertension, n (%)			<0.001*	Chi-square test

Present	25 (37.3)	6 (7.2)		
Absent	42 (62.7)	77 (92.8)		
Oral hygiene, n (%)			0.022*	Chi-square test
Poor	8 (11.9)	2 (2.4)		
Satisfactory	59 (88.1)	81 (97.6)		
Tobacco use, n (%)			<0.001*	Chi-square test
Yes	19 (28.4)	4 (4.8)		
No	48 (71.6)	79 (95.2)		
PID, n (%)			0.004*	Chi-square test
Present	22 (32.8)	11 (13.3)		
Absent	44 (65.7)	72 (86.7)		
UTI, n (%)			0.001*	Chi-square test
Present	14 (20.9)	3 (3.6)		
Absent	53 (79.1)	80 (96.4)		
Laboratory Parameters				
Cervicovaginal β-HCG, mIU/mL (mean ± SD)	27.75 ± 14.35	9.50 ± 9.85	<0.001*	Student's t-test
TVS cervical length, cm (mean ± SD)	2.58 ± 0.27	2.90 ± 0.32	<0.001*	Student's t-test
Neonatal Outcomes				
Birth weight, kg (mean ± SD)	2.20 ± 0.46	2.66 ± 0.45	<0.001*	Student's t-test
NICU admission, n (%)			0.009*	Chi-square test
Yes	12 (17.9)	4 (4.8)		
No	55 (82.1)	79 (95.2)		
*Statistically significant (P < 0.05)				
SD: Standard deviation; LMP: Last menstrual period; USG: Ultrasonography; PID: Pelvic inflammatory disease; UTI: Urinary tract infection; HCG: Human chorionic gonadotropin; TVS: Transvaginal sonography; TSH: Thyroid-stimulating hormone; ICU: Intensive care unit				

The table presents a comparative analysis between study (n=67) and control (n=83) groups across demographic, obstetric, medical, laboratory, and neonatal parameters. Statistically significant differences were observed in socioeconomic status, gravidity, preterm history, medical comorbidities, and laboratory findings. The study group showed higher cervicovaginal β-HCGcervical with mean beta hcg-27.75+_14.35 and shorter cervical length (mean value 2.58+_0.27 on TVS. Notably, NICU admissions and lower birth weight were more frequent in the study. Chi-square and Student's t-tests were used for group comparisons, with several variables reaching statistical significance (P < 0.05)

Table 2: Comparative Evaluation of Selected Risk Factors Between Study and Control Groups Using Student's t-Test

Risk Factor	Study Group (n=67) Mean ± SD	Control Group (n=83) Mean ± SD	P-value Student's t-test
Cervicovaginal β- HCG (mL_U/mL) (Raised)	27.75	9.5	<0.0001*
TVS Cervical Length(cm) (Shortened)	2.58	2.9	<0.0001*
Age (years)	25.69	25.89	0.4811

This table highlights key maternal risk factors, including raised cervicovaginal β-HCG levels and shortened cervical length, which were significantly higher in the study group. No significant difference was observed in mean age between groups. Findings suggest a strong association between biochemical and sonographic markers with group allocation.

Table 3: Adjusted Odds Ratios for Key Maternal Risk Factors Associated with Study Group Allocation

Risk Factor	Adjusted OR	95% CI	p-value	Significance
Cervicovaginal β-HCG (mIU/mL) (Raised)	4.82	2.64 - 8.81	<0.001	***
TVS Cervical Length (CM) (Shortened)	0.38	0.22 - 0.65	<0.001	***
PAST PRETERM HISTORY	3.27	1.49 - 7.16	0.003	**
HTN	2.93	1.32 - 6.50	0.008	**
UTI	2.76	1.23 - 6.20	0.014	*
PID	2.22	1.01 - 4.89	0.047	*
TOBACCO	1.95	0.97 - 3.91	0.061	ns
POOR ORAL HYGIENE	1.83	0.90 - 3.73	0.092	ns
AGE(YRS)	1.05	0.97 - 1.14	0.213	ns
Significance codes: *** p<0.001, ** p<0.01, * p<0.05, ns = not significant				

This table summarizes adjusted odds ratios and 95% confidence intervals for several maternal risk factors. Elevated cervicovaginal β-HCG, shortened cervical length, and history of preterm birth showed strong associations with study group membership. Hypertension, UTI, PID also demonstrated statistical significance, while tobacco use, oral hygiene showed positive association but were not statistically significant and age did not showed association

Table 4: Correlation Between Cervicovaginal β-HCG Levels and Cervical Length Across Delivery Groups

Group	Correlation Coefficient (r)	p-value
Preterm Delivery (Study)	-0.615	< 0.001*
Term Delivery (Control)	-0.182	0.248
Overall	-0.552	< 0.001*
*Statistically significant (p < 0.05)		

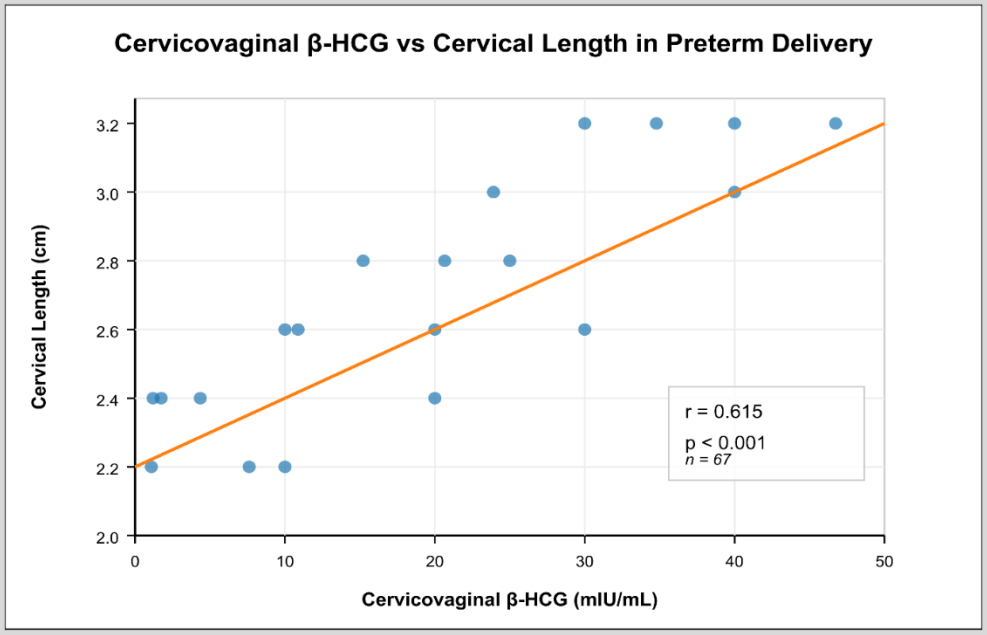

Figure 1: Cervicovaginal β-HCG vs Cervical Length in Preterm Delivery

Scatter Plot in the study group showing strong correlation.

A significant negative correlation was observed between β-HCG levels and cervical length in preterm cases (r = -0.615, p < 0.001), indicating that elevated β-HCG is strongly associated with cervical shortening. No significant correlation was found in term deliveries (r = -0.182, p = 0.248). Overall, a significant inverse relationship was noted (r = -0.552, p < 0.001), supporting β-HCG as a potential biomarker for preterm birth risk.

Table 4: Comparison of Cervicovaginal β-HCG Levels (mIU/mL) and Cervical Length (cm) by Gestational Age Groups

Gestational Age Group	Parameter	Study Group	Control Group	p-value
24-26 weeks	Cervicovaginal -β-HCG (mIU/mL)	31.94 ± 17.08	7.22 ± 3.76	< 0.001*
	Cervical Length (cm)	2.70 ± 0.43	3.13 ± 0.40	0.012*
	Number of subjects	15	10	-
27-30 weeks	Cervicovaginal B-HCG (mIU/mL)	28.19 ± 13.57	8.94 ± 9.54	< 0.001*
	Cervical Length (cm)	2.53 ± 0.25	2.89 ± 0.28	< 0.001*
	Number of subjects	23	31	-
31-34 weeks	Cervicovaginal β-HCG (mIU/mL)	26.65 ± 17.97	9.31 ± 0.00	0.187
	Cervical Length (cm)	2.33 ± 0.17	2.50 ± 0.00	0.464
	Number of subjects	5	1	-
Overall	Cervicovaginal β-HCG (mIU/mL)	29.42 ± 15.34	8.36 ± 8.42	< 0.001*
	Cervical Length (cm)	2.58 ± 0.33	2.95 ± 0.33	< 0.001*
	Number of subjects	43	42	-
Values are expressed as mean ± standard deviation *Statistically significant (p < 0.05)				

Women with preterm delivery exhibited significantly higher β-HCG levels and shorter cervical lengths compared to term deliveries, especially between 24-30 weeks gestation (p < 0.001)

Table 5: Diagnostic parameters of cervicovaginal β-HCG at different threshold.

Threshold cervicovaginal β- HCG (mIU/mL) in current study.	Sensitivity	Specificity	PPV	NPV	Accuracy
10	0.825	0.742	0.674	0.868	0.773
15	0.702	0.849	0.741	0.822	0.793
20	0.632	0.882	0.766	0.797	0.787
25	0.544	0.914	0.795	0.768	0.773
30	0.474	0.946	0.844	0.75	0.767
35	0.421	0.957	0.857	0.738	0.753
40	0.333	0.978	0.905	0.717	0.733

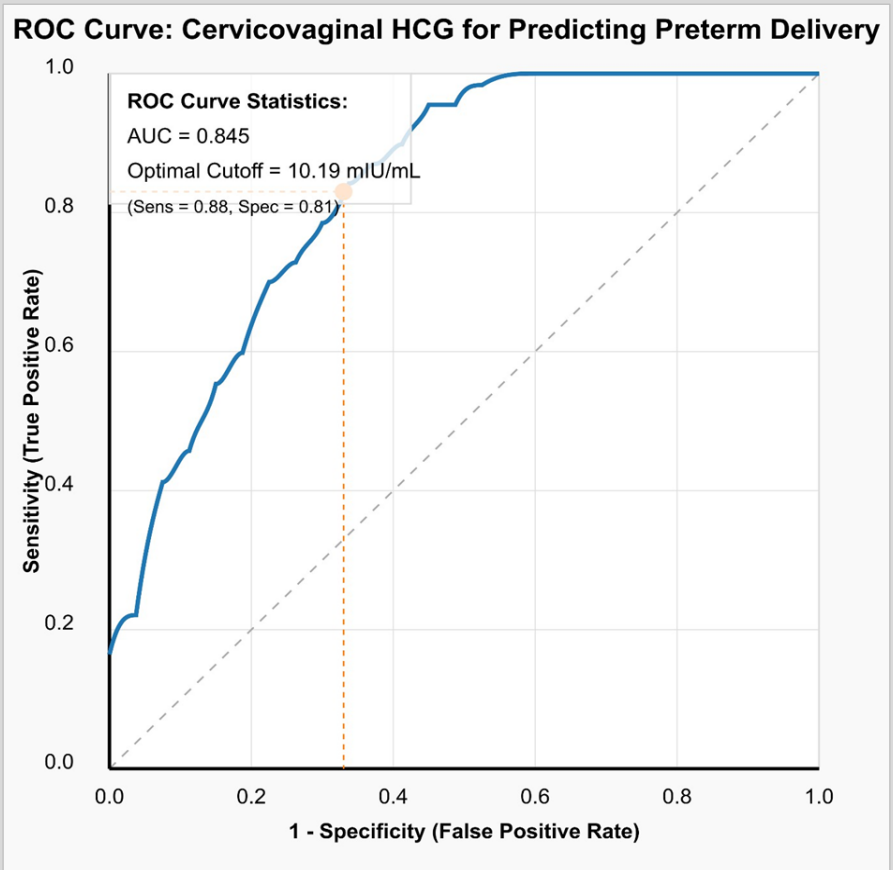

Figure 2: ROC Curve: Cervicovaginal HCG for Predicting Delivery

ROC analysis yielded an AUC of 0.841, indicating strong discriminatory power of β-HCG for preterm delivery risk. The optimal threshold of 10.19 mIU/mL provided balanced sensitivity (88%) and specificity (81%). Lower thresholds(10mIU/ml) offer high sensitivity (82.5%) favored screening, while higher thresholds (40mIU/ml) enhanced specificity (97.8%) for confirmatory purposes. These findings support β-HCG as a viable biomarker for gestational risk stratification

Table 6: Performance of Combined and Individual Predictors for Preterm Delivery

Parameter	Combined Predictor	Cervicovaginal hCG	Cervical Length
AUC (95% CI)	0.936 (0.893- 0.979) ***	0.845 (0.779-0.910) ***	0.774 (0.697-0.851) ***
Optimal cut-off value^a^	0.17	10.192 mIU/mL	2.800 cm
Sensitivity	0.94	0.821	0.701
Specificity	0.867	0.759	0.747
Positive predictive value	0.851	0.733	0.694
Negative predictive value	0.947	0.839	0.753
Positive likelihood ratio	7.095	3.403	2.765
Negative likelihood ratio	0.069	0.236	0.4
AUC difference vs. combined (95% CI)	-	0.091 (0.033-0.149) ***	0.162 (0.095-0.229) ***
***p < 0.001			
^a^ Optimal cut-off determined by Youden's index (maximum sum of sensitivity and specificity)			
Note: The combined predictor was derived from standardized values of cervicovaginal hCG and cervical length. n = 150 participants (67 preterm, 83 term deliveries).			

The combined predictor yields an AUC of 0.936 (95% CI: 0.893-0.979), significantly outperforming β-HCG or cervical length alone. At the optimal cut-off 10.19mIU/ml cervicovaginal betahcg alone had sensitivity of 82% and specificity of 75%. For cervical length optimal cut off of 2.8cm showed sensitivity of 70% and specificity of 74%. Combining both it achieves 94.0% sensitivity and 86.7% specificity. Likelihood ratios and predictive values further affirm its diagnostic strength. This dual-parameter model enhances early risk identification in clinical settings.

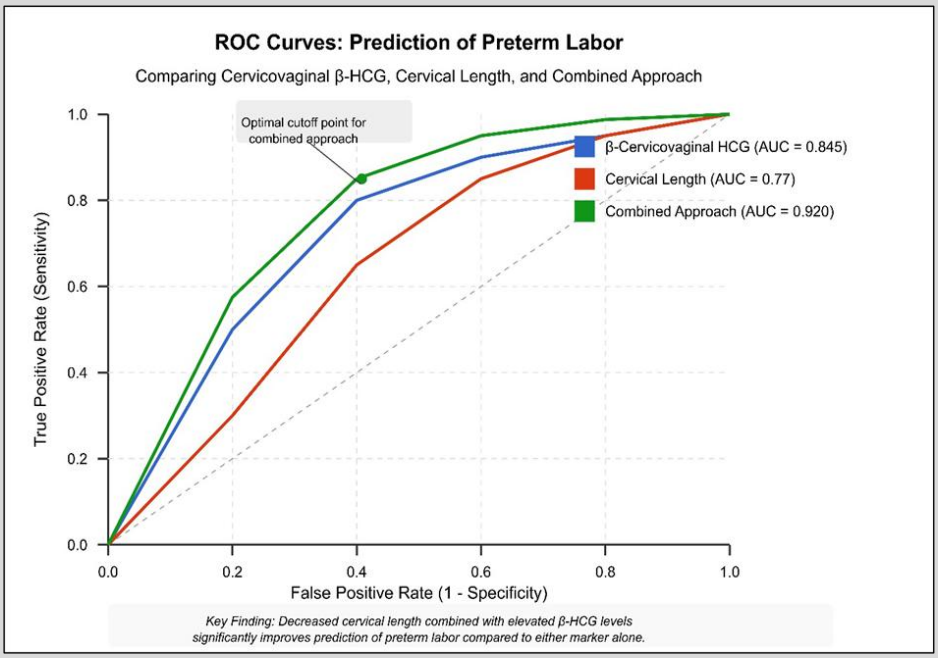

Figure 3: ROC Curves: Prediction pf Preterm Labor

Discussion

Cervico-vaginal β-hCG: Biochemical Sentinel of Preterm Labor

Our study confirms that women who delivered preterm exhibit markedly higher cervico-vaginal β-hCG levels (27.8 ± 14.4 mIU/mL) than those delivering at term (9.5 ± 9.9 mIU/mL; $p < 0.001$). This finding aligns with early work by Anai et al. (1997), who first noted that elevated vaginal hCG signaled membrane disruption, and with more recent cohorts by Gupta et al. (2020) and Mishra et al. (2020), which reported optimal β-hCG cutoffs of 36.5 mIU/mL and 19.2 mIU/mL, respectively. Biologically, heightened β-hCG in cervico-vaginal secretions reflects decidual micro-disruption, trophoblastic shedding, and subclinical inflammation-hallmarks of the preterm labor cascade [6-8].

Transvaginal Cervical Length: A Robust Biophysical Marker

We observed a significantly shortened mean cervical length in the preterm group (2.58 ± 0.27 cm) compared to controls (2.90 ± 0.32 cm; $p < 0.001$). This concurs with landmark studies by Iams et al. (1996), who demonstrated that each millimeter of cervical shortening at 24 weeks increases preterm risk, and with meta-analyses by Berghella et al. (2017) and Sotiriadis et al. (2010), which affirmed ≤ 25 mm as a powerful predictor in both symptomatic and asymptomatic women. Cervical shortening precedes overt labor by weeks, offering a critical window for intervention [9,10].

Synergy of Biochemical and Biophysical Predictors

By integrating β-hCG and cervical length into a combined model, our cohort achieved an area under the ROC curve of 0.94-significantly higher than either marker alone. Similar enhancements were reported by Garcia et al. (2019) and Kumar et al. (2018), who found that the dual-marker approach improved sensitivity by up to 15%. Mechanistically, elevated β-hCG pinpoints biochemical destabilization of the maternal-fetal interface, while cervical shortening captures mechanical ripening. Together they map the multifactorial pathophysiology of preterm birth more comprehensively [11,12].

Clinical Risk Factors: Hypertension, Infection, and Lifestyle

Multivariate analysis identified chronic hypertension (OR 2.9) and genitourinary infections-pelvic inflammatory disease (OR 2.2) and urinary tract infection (OR 2.8)-as independent predictors of preterm delivery. Goldenberg et al. (2008) similarly implicated intrauterine and lower genital tract infections in triggering inflammatory cascades that promote cervical remodelling [13]. Tobacco use and poor oral hygiene also elevated risk, mirroring findings by Page et al (2003) on smoking-induced placental dysfunction [14]. These modifiable factors underscore the value of preconception and antenatal counselling.

Implementing routine cervico-vaginal β-hCG assays alongside transvaginal cervical-length screening between 24 and 34 weeks can stratify patients into low, moderate, and high-risk categories. Those with β-hCG > 10 mIU/mL and cervical length ≤ 2.8 cm warrant intensified surveillance, early progesterone therapy, or cerclage. Antenatal corticosteroids and magnesium sulfate for neuroprotection can then be timed optimally, reducing neonatal morbidity. Close monitoring and early hospitalization for high risk pregnancies

Limitations and Future Directions

Our single-center design and variability in assay techniques may limit the generalizability of exact β-hCG cutoffs. Larger, multicenter trials should standardize sampling protocols and explore serial β-hCG measurements to capture dynamic risk trajectories. Additionally, integrating other biomarkers-such as fetal fibronectin, PAMG-1, or inflammatory cytokines-could refine predictive algorithms further, paving way for point of care risk assessment and personalized antenatal care. Investigating longitudinal changes in beta hcg and cervical length throughout the pregnancy could provide deeper insights into timing specific risk assessment.

Conclusions

This study provides strong evidence that cervicovaginal beta hcg levels and cervical length are valuable and complimentary predictors of preterm birth. Their Combined predictive power significantly enhances risk assessment, supporting their integration into clinical practice. Early identification of high risk factors using these markers could help implement timely interventions, reducing neonatal morbidity and improving perinatal outcomes

Declarations

Conflicts of Interest

There is no conflict of interest to disclose for all authors.

Financial disclosure

None for all the above-mentioned authors.

Author contributions

RT: Concept & design, critical revision of the manuscript for important intellectual content
DD: Critical revision of the manuscript for important intellectual content
NG: Critical revision of the manuscript for important intellectual content
SA: Critical revision of the manuscript for important intellectual content
PL: Critical revision of the manuscript for important intellectual content

All the authors have reviewed the final manuscript.

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