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Beta HCG in cervico-vaginal secretion as a predictor of preterm labor

Ahmed M Radwan[1*], Marwa A Fayez[2]
[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Sharkia, Egypt
[2] Department of Clinical Pathology, United Doctors Hospital, Jeddah, Saudi Arabia

Abstract
Background: elevated B-hCG level in cervicovaginal secretion may be considered as a marker to predict preterm labor.
Aim: Aim of this prospective study was to assess the correlation between cervicovaginal levels of B-hCG and preterm labor.
Methods: 120 pregnant women at gestational age 28-36 weeks, including 40 as control (group II) having no preterm labour pain and 80 in the study group having preterm labour pain of which 40 had preterm delivery (group Ia) and 40 had term delivery (group Ib).
Results: The median of B-hCG level (mIu/ml) in group IA was 24.5(14-44.5), in Ib was 7.6(6-24.5), in II was 3.6(2.35-4.5), ($p<0.001$). The cut off value of β-hCG >5.8 mIU/ml in cervicovaginal secretion can be used to predict preterm delivery in women with preterm labor pain.
Conclusions: β-hCG level in cervico-vaginal secretion can be used as a predictor of preterm labor in women presenting with preterm labor pain, with cut off value > 5.8 mIu/ml.

Keywords: B-HCG, cervico-vaginal, secretions

Introduction
Preterm birth is defined when delivery happens before completing Gestational age of 37 weeks. This definition has been used since 1976 and for more than 45 years by the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (FIGO) [1]. As long as the neonatal morbidities and mortalities are increasing with decreasing gestational age at delivery, the preterm births are subdivided into two subgroups; births before 34 weeks are called early preterm births and those occurring between 34 and 36 weeks are called late preterm births carrying less neonatal complications [2].
Guarding against neonatal morbidities and mortalities necessitates prediction of preterm births. Many researchers studied various biochemical markers as a prediction of preterm birth, such as fetal fibronectin, maternal serum Alfa fetoprotein (MSAFP), salivary estriol and cervicovaginal B-hCG [3, 6].
Human chorionic gonadotropin is a glycolproteins which presents in maternal serum and amniotic fluid during pregnancy [7]. Elevated levels of B-hCG in cervicovaginal secretions may reflects the inflammatory reactions preceding the onset of labor [8].
Our aims in this study were to assess the correlation between cervicovaginal levels of B-hCG and preterm labor, and to detect a definitive cut-off value of cervicovaginal B-hCG to predict preterm labor.

Materials and Methods
This was prospective cohort study. It was done in Department of Obstetrics and Gynecology at United Doctors Hospital, Jeddah, Saudi Arabia. Pregnant women included in this study were at the gestational age between 28 and 36 weeks. They were recruited from Antenatal Clinic and Emergency with or without labor pain, from March 2018 to February 2019. The gestational age was calculated by the first day of the last menstrual period (LMP) and confirmed by ultrasound. If the difference between the gestational age by (LMP) and ultrasound was more than ten days, the reported age by ultrasound was considered as the gestational age. Whenever the woman was unsure of her (LMP), the gestational age was determined by ultrasound.

Criteria of Inclusion
All pregnant women in this study were at the gestational age between 28-36 weeks, singleton confirmed by early second trimester ultrasound), cervical dilatation less than 3 cm, intact amniotic membranes (with no leakage of amniotic fluid; negative Amnicator® test), non-existence of any maternal and fetal complications during pregnancy.

Criteria of Exclusion
Any form of bleeding with pregnancy (placenta previa and abruptio placenta), hypertensive women, diabetic women, placental insufficiencies, fetal distress, fetal growth disorders (fetal growth restriction and macrosomia), fetal congenital anomalies and multiple gestations.
This study included 120 pregnant women and were divided into two groups; 80 in study group and 40 in control group.

Group-I; Study Group
80 women with preterm labor pain, given tocolysis and followed till delivery. This group was subdivided into two subgroups: -

Study Group IA: 40 pregnant women with preterm labor given tocolysis but proceed to preterm delivery.
Study Group Ib: 40 pregnant women with preterm labor pain given tocolysis and delivered at full term.

Group-II; Control Group:
40 pregnant women with no preterm labor pain and followed till delivery.
Sample collection and biological principles of the procedure:
Cervicovaginal secretions were collected before per-vaginal examination and before treatment with tocolysis by placing a cotton tipped swab into endocervix and then in posterior vaginal fornix for 30 seconds. The swab was shaken with 1 ml saline for 1 minute followed by 5 minutes centrifugation at rate of 1500 rpm. The ARCHITECT total B-hCG assay is a two-step immunoassay to determine the presence of B-hCG in human serum, plasma and secretions using Chemiluminescent Microparticle Immunoassay(CMIA)technology with flexible assay protocols, referred to as Chemiflex machine used is ARCHITECT c4000©2016Abbott park, Illinois, U.S.A.

Details of Statistical Tests
Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The distribution of quantitative data was tested by Kolmogorov-Smirnov test of normality. Quantitative data were presented as mean and standard deviation when parametric and compared between two groups using Independent t-test and between three groups using One Way ANOVA while non-parametric were presented as median with inter-quartile range (IQR) and compared between two groups using Mann-Whitney test and between three groups using Kruskall-Wallis test. Also, qualitative variables were presented as number and percentages and compared between groups using Chi-square test. Spearman correlation coefficients were used to assess the correlation between β-hCG level and the other studied parameters. Receiver operating characteristic curve (ROC) and logistic regression were used to assess the validity of β-hCG level to predict pre labor rupture of membrane. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of < 0.05.

Results
Out of a total of 120 pregnant women recruited in this study, 40 (33.3%) were controls, while 80 (66.6%) were cases among which a total of 40 (33.3%) were in Study Group Ia and remaining 40 (33.3%) were in Study Group Ib. (Figure 1

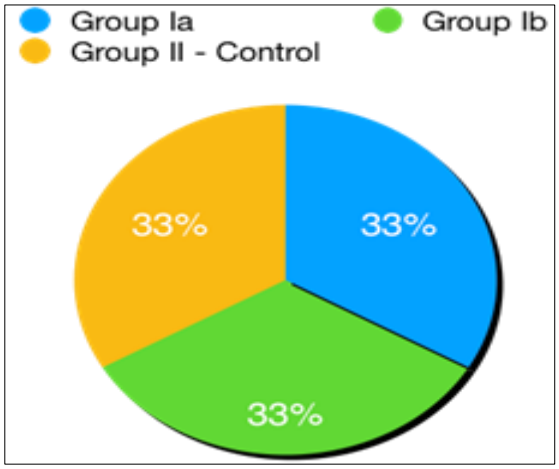

Fig 1: Distribution of subjects

As shown in (Table 1), the mean age ±SD in study group Ia was 26.32+/-3.84, group Ib was 24.81+/-4.21 and control group was 25.62+/-3.41. All the 120 women included in this study were in lower and middle socioeconomic class. The parity range of most of the women in study group Ia and Ib were 2-3 while in control group most were 1-3. Body mass index (BMI) of women in control group lie in the range of 24.61+/-4.62 kg/m2, and in the range of 24.68+/-4.40 kg/m2 in study group I. There was no statistically significant difference between study and control groups with respect to age (p=0.947), socioeconomic status (p=0.893), parity (p=0.786) and BMI (p=0.936). However, the difference of β-hCG level (35.37+/-3.49 mIu/ml in group I and 37.56+/-4.36 mIu/ml in group II) with Gestational age (in weeks) at which sample taken was statistically significant (p=0.004). The B-hCG level, median (IQR) in the study group was 15.35(6.7-33.6) mIu/ml, while it was 3.6(2.35-4.5) mIu/ml, making a significant statistical difference (p<0.001) (Figure 2).

Table 1: Maternal data and level of β-hCG among the studied groups; Group I & Group II

Maternal data	Group I No. = 80	Group II No. = 40	Test value	P-value
Age (year), mean± SD	25.57±4.03	25.62±3.41	0.067•	0.947
BMI (kg/m^2), mean± SD	24.68±4.40	24.61±4.62	0.081•	0.936
GA (weeks) at delivery, mean± SD	35.37±3.49	37.56±4.36	2.976•	0.004
Parity, median (IQR)	2 (0 – 3)	2 (1 – 3)	0.832≠	0.786
Gravidity, median (IQR)	1 (0 – 2)	0 (0 – 1)	1.042≠	0.618
Socioeconomic, no. (%)			0.018*	0.893
Low	49 (61.3%)	25 (62.5%)		
Middle	31 (38.8%)	15 (37.5%)		
β-hCG level (mIU/ml), median (IQR)	15.35 (6.7 – 33.6)	3.6 (2.35 – 5.4)	6.317≠	<0.001

•: Independent t-test; ≠: Mann-Whitney test; *: Chi-square test

BMI: Body Mass Index; GA: Gestational age; HCG: Human chorionic gonadotropin

Bold indicate significant difference between groups

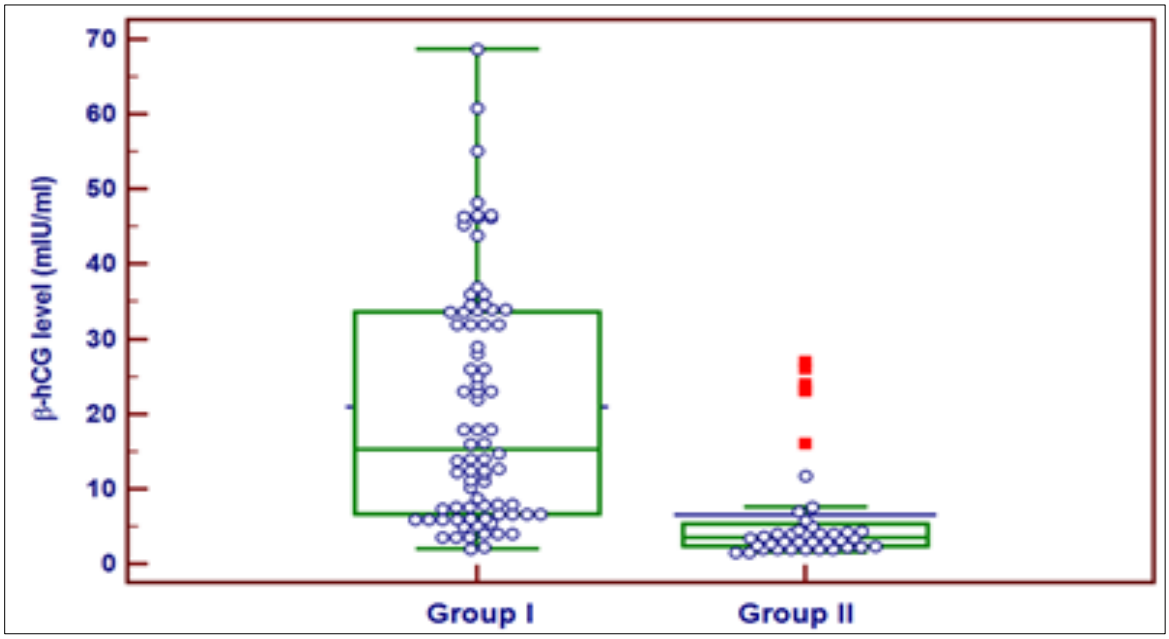

Fig 2: B-hCG levels (mIu/ml) in Group I and Group II.

Table 2 shows no statistically significant difference between study group Ia, study group Ib, and control group II with respect to age (p=0.216), socioeconomic status (p=0.512), parity (p=0.642) and BMI (p=0.662). The only statistical significant difference was reported in β-hCG level (32.12+/-2.35 mIu/ml in group Ia, 38.61+/-4.62 mIu/ml in group Ib, and 37.56+/-4.36 mIu/ml in group II) with Gestational age (in weeks) at which sample taken (p<0.001). The B-hCG level, median (IQR) in group IA was 24.5(14-44.5) mIu/ml, in group Ib was 7.6(6-24.5) mIu/ml, while it was 3.6(2.35-4.5) mIu/ml, making a significant statistical difference (p<0.001) (Figure 4).

Table 2: Maternal data and level of β-hCG among the studied groups

Maternal data	Group Ia No. = 40	Group Ib No. = 40	Group II No. = 40	Test value	P-value
Age (year), mean± SD	26.32±3.84	24.81±4.21	25.62±3.41	1.554•	0.216
BMI (kg/m^2), mean± SD	24.22±4.81	25.13±3.98	24.61±4.62	0.415•	0.662
GA (weeks) at delivery, mean± SD	32.12±2.35^a	38.61±4.62^b	37.56±4.36^b	31.744•	<0.001
Parity, median (IQR)	2 (0 – 3)	3 (1 – 3)	2 (1 – 3)	1.325≠	0.642
Gravidity, median (IQR)	1 (0 – 2)	1 (0 – 1)	0 (0 – 1)	0.865≠	0.821
Socioeconomic, no. (%)				1.340*	0.512
Low	27 (67.5%)	22 (55.0%)	25 (62.5%)		
Middle	13 (32.5%)	18 (45.0%)	15 (37.5%)		
β-hCG level (mIU/ml), median (IQR)	24.5 (14–44.5) ^{a}	7.6 (6 – 24.5) ^{b}	3.6 (2.35 – 5.4) ^{c}	52.451≠	<0.001

•: One Way ANOVA followed by post hoc test using LSD when significant; ≠: Kruskall-Wallis test followed by post hoc test using Mann-Whitney test when significant; *: Chi-square test

BMI: Body Mass Index; GA: Gestational age; HCG: Human chorionic gonadotropin

Bold indicate significant difference between groups

Different superscript letter indicates significant difference regarding post hoc analysis

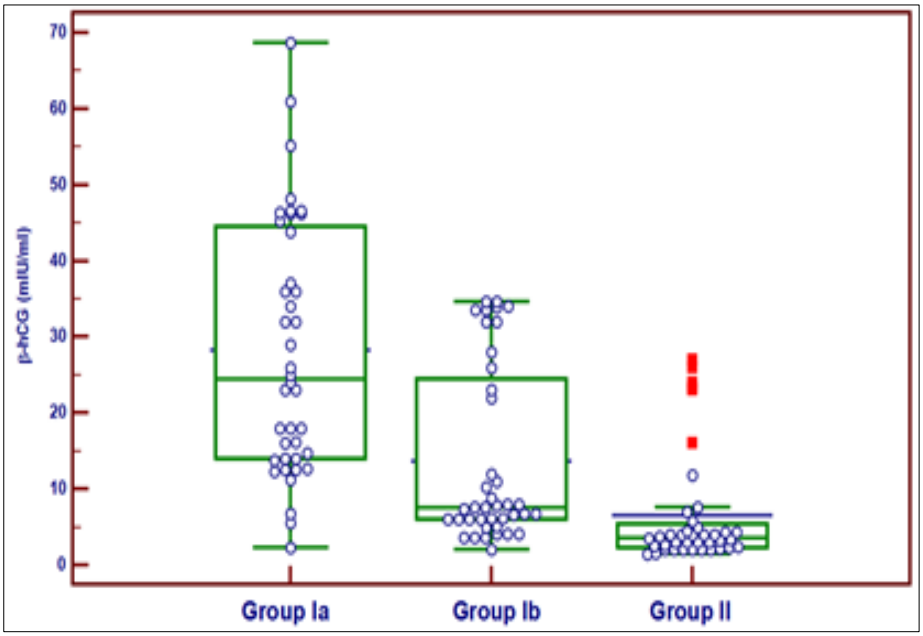

Fig 3: B-hCG levels (mIu/ml) in Group Ia, Group Ib, and Group II.

Table 3 reports insignificant and negative correlation of B-hCG level with age (r=-0.222, p=0.158) and with parity (r=-0.068, p=0.67). The correlation of B-hCG levels with BMI (kg/m2) and gravidity was positive but insignificant; (r=0.061, p=0.702) and (r=0.079, p=0.618) respectively. The correlation of B-hCG level with gestational age (in weeks) at which sample taken was negative and significant (r= -0.758, p<0.001) (Figure 4). Then. for predicting cases with β-hCG level, the Gestational age at which sample taken should be required to include in the model.

Table 3: Correlation of β-hCG level with the other studied parameters

	β-hCG level (mIU/ml)	
	r	**p-value**
Age (year)	-0.222	0.158
BMI (kg/m2)	0.061	0.702
GA (weeks)	-0.758**	0.000
Parity	-0.068	0.67
Gravidity	0.079	0.618

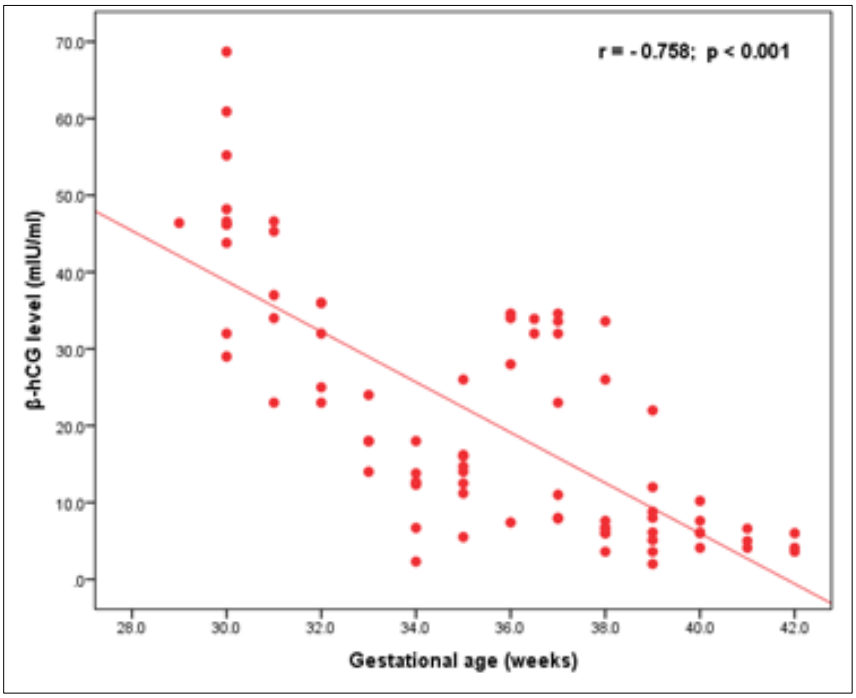

Fig 4: correlation of β-hCG level (in mIu/ml) with Gestational age (in weeks) at which sample taken; (r=-0.758, p<0.001)

Receiver operating characteristic curve (ROC) for β-hCG level in differentiation between groups; A: group I versus group II, B:

group Ia versus group Ib, C: group Ia versus group II, and D: group Ib versus group II (Figure 5) is illustrated in (Table 4). The ROC for B-hCG level in differentiation shows sensitivity of (86.25, 92.50, 92.50, and 90.00) and specificity of (77.50, 67.50, 82.5, 65.00) between groups (A: group I versus group II, B: group IA versus group Ib, C: group Ia versus group II, and D: group Ib versus group II) respectively.

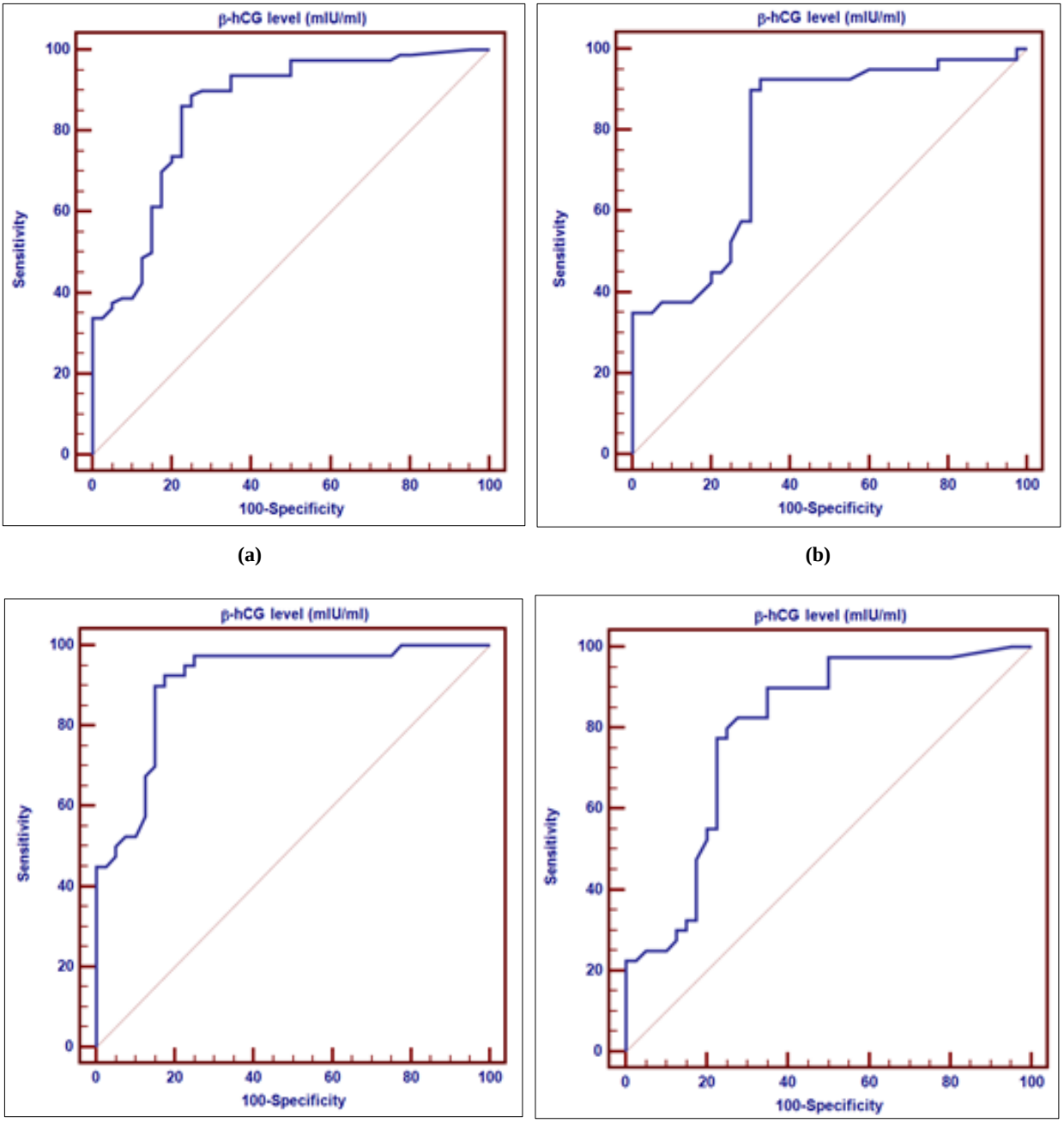

Fig 5: Receiver operating characteristic curve (ROC) for β-hCG level in differentiation between groups

Table 4: Receiver operating characteristic curve (ROC) for β-hCG level in differentiation between groups

Groups	Cut off	AUC	Sensitivity	Specificity	PPV	NPV
A: Group I vs group II	>5.8 *	0.855	86.25	77.50	88.5	73.8
B: Group Ia vs group Ib	>11	0.789	92.50	67.50	74.0	90.0
C: Group Ia vs group II	>7.6	0.909	92.50	82.50	84.1	91.7
D: Group Ib vs group II	>4	0.800	90.00	65.00	72.0	86.7

AUC: Area under curve; PPV: Positive predictive value; NPV: Negative predictive value

The logistic regression analysis for B-hCG level between groups was significant; group I versus group II ($p<0.001$), group IA

versus group II (p<0.001), group Ib versus II (p=0.005), and group Ia versus Ib (p=0.0002) as reported in (Table 5).

Table 5: Logistic regression analysis for β-hCG level between groups

β-hCG level (mIU/ml)	P-value	OR	95% CI
Group I vs Group II	<0.001	1.128	1.064 to 1.196
Ia vs II	<0.001	1.177	1.095 to 1.126
Ib vs II	0.005	1.0822	1.025 to 1.143
Ia vs Ib	0.0002	1.0744	1.035 to 1.115

OR: Odds ratio; CI: Confidence interval Bold indicates significant

Discussion

In this study we compared B-hCG value in cervicovaginal secretions in women with preterm labor and with normal pregnancy and we evaluated its cut-off value to predict preterm labor.

This may be useful for early detection of asymptomatic preterm, and helpful in preventing complications of preterm labor and its fetal hazards; including fetal lung immaturity and fetal neurological complications. Also, this will decrease the intervals of follow up visits and hospital stay [9].

The first study considered measuring levels of vaginal fluid B-hCG as a useful marker of premature rupture of membranes during second and third trimesters of pregnancy was done by Anti *et al* [10].

In our study, the difference in the mean age between study and control group was statistically insignificant. However, lower socioeconomic status predisposes to preterm labor which was like the study by RL Weck [11].

We reported in this study statically insignificant difference between increased parity and occurrence of preterm labor which was like the study done by Ranjbar M. *et al*. [12].

However, Shaikh *et al* reported increased parity as a risk factor for preterm labor, and common complications of multiparity like placenta previa and abruptio placenta may carry higher risk of preterm labor [13].

Although the study done by Dayton Shin and Won O. Song concluded that women with BMI<19kg/m2 have 25.7 % chance of preterm labor and 10.1 chance of preterm delivery, we did not find in our study an association between BMI and preterm delivery [14].

We found in our study statistically significant rise in the level of cervicovaginal B-hCG in the study group with median and interquartile range (IQR) of 15.35 (6.7 – 33.6) mIU/ml as compared to the control group with median (IQR) of 3.6 (2.35 – 5.4) mIU/ml. Among the study group, statistically significant difference was reported with group IA with median (IQR) of 24.5 (14 – 44.5) mIU/ml having higher level of cervicovaginal B-hCG than group Ib with median (IQR) of 7.6 (6 – 24.5) mIU/ml (p<0.001). These findings were similar to study done by Mishra N *et al* which documented mean value of 23.46±8.86 SD mlU/ml in the study group and 7.81 ±16.19 SD mlU/ml in control group, concluding that mean level of B-hCG in study group was statistically significantly higher than in control group (P<0.0001) [15].

In present study, the only significant and positive correlation of B-hCG level was found with gestation at which sample taken (r=-0.758, p<0.0001). Study group Ia (preterm labor and preterm delivery group) had significantly higher mean B-hCG levels as compared to study group Ib (preterm labor and term delivery group) (32.12+2.35 vs. 38.61 ±3.98 mIU/ml respectively, P<0.001) and the optimal cut- off β-hCG for predicting the preterm delivery using β-hCG levels only was ?? MIU/ml with sensitivity 86.25%, specificity 77.5%, positive predictive value 88.5% and negative predictive value 73.8%.

Similar findings were reported by Sak *et al*. as significantly higher cervicovaginal B-hCG levels (94.7±37.7mIU/ml) were found in women with preterm labour compared to levels in those with term labour (35.5±14.8 mIU/ml, P<0.001) and the optimal cut-off B-hCG level value of 75 mIU/ml, with sensitivity of 76%, specificity 91.6%, positive predictive value 95% and negative predictive value 79.9% [16].

Another cohort study by Garshabi *et al* included 540 pregnant women to measure B-hCG levels in cervicovaginal secretions between 20- and 28-weeks' gestation and reported similar conclusion. They found that single measurement of cervicovaginal B-hCG higher than 77.8 mI/ml is a sensitive 87.5% (95% CI: 47.4-97.9) and specific 97% (95% CI: 86.5-99.4) predictor of preterm labor. This was an independent predictor as indicated by multiple logistic regression (adjusted odds ratio 19.97, 95% CI: 10.65-37.45) [17].

A study by Adhikari *et al* concluded that cervicovaginal level of B-hCG higher than 14 mIU/ml is a useful predictor of delivery before 34 weeks, with sensitivity 83.3%, specificity 85.5%, PPV 33.3% and NPV 98.3 [18].

Bahasadri *et al*. in a cross-sectional study including 123 women reported that vaginal fluid β-hCG was higher in women with preterm premature rupture of membranes and who were suspected to than those with intact membranes. Thus, evaluation of vaginal fluid B-hCG may be a suitable, fast and reliable test to predict and diagnose rupture of membranes [19].

Another similar study by Esim *et al* (2003) [20] including 141 patients reported significantly higher level of vaginal fluid β-hCG in women with confirmed premature rupture of membranes than reported in women suspected or unconfirmed premature rupture of membranes and women with normal term delivery. They recommended determination of vaginal B-hCG level as a simple reliable rapid test to diagnose premature rupture of membranes [20].

Bagga *et al* (2010) [21] studied both cervical length and cervicovaginal β-hCG as predictors of preterm delivery. They found higher level of cervico-vaginal β-hCG in preterm delivery than found in term delivery [21].

Conclusion

This study found higher levels of B-hCG in cervicovaginal secretions in women with preterm labour pain and had preterm delivery than in women with preterm labour pain with term delivery and normal pregnancy with no preterm labor pain.

We can use cut off value of β-hCG >5.8 mIU/ml in cervico-vaginal secretion to predict preterm delivery in patients presenting with preterm labor.

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