Original Article

Vitamin D-Binding Protein in Cervicovaginal Fluid as a Non-Invasive Predictor of Maternal and Fetal Outcome in Women with Preterm Labor

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ABSTRACT

Aim: This study aimed to determine whether vitamin D-binding protein (VDBP) in cervicovaginal fluid (CVF) is independently predictive of imminent spontaneous preterm delivery (SPTD, delivery within 48 hours) in women with preterm labor with intact membranes (PTL).

Materials and Methods: This was a single-center observational cohort study. CVF samples for VDBP assays were obtained along with serum C-reactive protein (CRP) levels in consecutive women with PTL (n = 94) between 23.0 and 37.0 weeks of gestation. VDBP levels in CVF were determined by enzyme-linked immunosorbent assay kits. The primary outcome measures were SPTD within 48 hours after sampling.

Results: In the multivariable analysis, elevated VDBP levels in CVF samples of PTL women were significantly associated with imminent preterm delivery, even after adjusting for potential confounders (e.g., gestational age at sampling, parity, and serum CRP). In women with PTL, the areas under receiver operating characteristic curves of CVF VDBP level for predicting imminent preterm delivery were 0.781, with cut-off values of 2.3 μg/mL (sensitivity of 63.16% and specificity of 96.0%), respectively. The CVF VDBP levels were significantly high in women with PTL.

Conclusion: VDBP in the CVF independently predicts imminent preterm delivery in women with PTL.

Key Words: Cervicovaginal fluid, maternal and fetal outcome, non-invasive predictor, preterm labor, vitamin D-binding protein

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INTRODUCTION

Spontaneous preterm labor and intact membranes (PTL) or spontaneous preterm premature rupture of the membranes (PPROM) accounts for approximately 70 to 80% of all preterm births, and preterm birth, owing to these causes in particular, is strongly associated with significant neonatal morbidity, mortality, and long-term disability[4]. Evidence suggests that the impact of preterm birth on adverse neonatal outcomes is directly related to the degree of prematurity and the occurrence of subclinical intra-uterine infection[2]. Therefore, the ability to predict the risk of spontaneous preterm delivery (SPTD) and intra-uterine infection more precisely, especially using non-invasive methods, has important clinical implications in terms of the treatment strategy (e.g., administration of medications [i.e., corticosteroid, antibiotics, and magnesium for neuroprotection] and transfer to a tertiary center) and the counseling of patients with PTL or PPROM.

Traditionally, measurement of inflammatory biomarkers in amniotic fluid (AF) sample obtained by amniocentesis has been extensively used for the prediction of intra-amniotic infection and SPTD. However, this measurement is currently limited in clinical practice due to the requirement of invasive AF sampling. In this context, cervicovaginal fluid (CVF), which can be obtained via non-invasive or minimally invasive methods, is a feasible alternative to the AF, because several studies have demonstrated changes in various inflammatory proteins present in the CVF in association with intra-amniotic infection/inflammation, premature ripening, cervical dilatation, and preterm birth[10].

Vitamin D-binding protein (VDBP) is a 58-kDa protein of the albumin superfamily that is mainly synthesized by hepatocytes. The established functions of VDBP include acting as a major carrier protein for vitamin D and its metabolites in serum, sequestering actin, and potentially modulating the inflammatory and immune response, and it is associated with the clinical progression of many diseases[5]. In particular, previous studies by[13] and[7] that used the proteomic and cohort approaches have shown significantly increased expression of CVF VDBP in

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association with the occurrence of impending PPROM in asymptomatic women and of SPTD and intra-amniotic infection in women presenting with symptoms of PTL.

However, these findings have not been confirmed by other studies. Moreover, whether the change in VDBP level in the CVF is associated with intra-amniotic infection and impending SPTD in women with PPROM remains unclear. Hence, the aim of this study was to determine whether the level of VDBP in CVF samples is independently predictive of intra-amniotic infection and SPTD within 48 hours in women with PTL or PPROM.

AIM OF THE WORK

This study aims to assess accuracy of Vitamin D-binding protein in cervicovaginal fluid in predicting spontaneous preterm delivery.

PATIENTS AND METHODS

Study design: An observational cohort study.

Study Setting: Patients attending Ain-Shams University Maternity Hospital.

Study Population: This observational study consisted of consecutive singleton pregnant women diagnosed with spontaneous PTL (n = 94) at 23 +0 to 37 +0 weeks of gestation from December 2018 to July 2019.

Sampling Method

The CVF samples will be collected from the posterior vaginal fornix using two sterile Dacron swabs (UTMtm, Copan Italia S.p.a., Italy) placed for 15 seconds to absorb the cervicovaginal secretions under sterile speculum examination. The two Dacron swabs were then placed in two cryotubes each containing sample buffer and stored at -70°C for further analysis.

Serum C-reactive protein (CRP) level was usually measured within 2-3 hours of sampling using latex-enhanced turbidimetric immunoassay (Denka Seiken, Tokyo, Japan), according to hospital protocol.

The CVF VDBP levels were measured using an enzyme-linked immunosorbent assay Human VIDAS Kit (bioMérieux, VIDAS, France).

All the patients will be subjected to:

1-History taking: Personal history, menstrual history, detailed obstetric history, history of any medical illness.

2-Physical examination: general, abdominal and pelvic as indicated.

3-CVF samples: The CVF samples were collected from the posterior vaginal fornix using two sterile Dacron swabs.

4-Laboratory investigation: CRP.

5-Gestational age was calculated based on the last menstrual period and the first trimester or second trimester (≤20 weeks) ultrasound results, when available.

Inclusion criteria:

- Alive fetus will be delivered.
- An aliquot of CVF sample available for analysis.
- Regular contraction of uterus, with a frequency of at least two contractions every 10 minutes resulting in changes in cervix that start before 37 weeks of pregnancy.
- Changes in cervix include effacement and dilation.

Exclusion criteria:

- Multiple pregnancies.
- Major fetal congenital anomalies.
- Prior cervical cerclage.
- Evidence of clinical chorioamnionitis at the time of presentation.

Outcome measure: A serum CRP and SPTD within 48 hours of sampling.

STATISTICAL ANALYSIS:

Data were analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY). Multivariable logistic regression was used to identify determinants of spontaneous PTL within 48 h. Two-sided *p-values <0.05* were considered statistically significant.

RESULTS

The results showed statistically significant difference between ≤48 hrs and >48hrs according to parity, GA at delivery (weeks) and highly statistically significant difference between ≤48 hrs and >48hrs according to serum CRP (mg/dL) and cervicovaginal VDBP (Table 1).

Moreover, the study revealed receiver-operating characteristic curves for vitamin D-binding protein in the cervicovaginal fluid and serum CRP in the prediction of imminent preterm delivery within 48 hours (AUC 0.781, *P 0.002*, SE 0.075, best cut-off criterion is >2.3 µg/ml and AUC 0.971, *P 0.001*, SE 0.027, best cut-off criterion is CRP >5.7 mg/l respectively, in women with preterm labor and intact membranes (Table 2).

The data of table 3 showed comparison of receiver-operating characteristic (ROC) curves for prediction of spontaneous PTL within 48 hours using serum CRP or cervicovaginal VDBP (AUC = 0.971 versus 0.781, respectively, difference = 0.189, SE 0.068, 95% CI 0.057 to 0.322, significance level 0.005).

So, serum CRP had better predictsive value compared with cervicovaginal VDBP.

Moreover, table 4 showed the correlation between serum CRP level and cervicovaginal VDBP which is moderate positive correlation between both variables (r = 0.407, *P-value < 0.001*). Also, it showed the correlation between serum CRP level and GA at delivery which is strong negative correlation between both variables (r = -0.745, *P-value < 0.001*).

Table 1: Comparison of patients who underwent spontaneous PTL within 48 hours or after 48 hours

Variable	SPTL > 48 h (n=75)	SPTL ≤ 48 h (n=19)	Difference	95% CI	*P-value**
Age (years)	31.6 ± 4.9	30.46 ± 4.7	-1.2	-3.7 to 1.3	0.338
Parity					0.016#
Multipara	61 (81.3%)	10 (52.6%)	-	-	
Nullipara	14 (18.7%)	9 (47.4%)	-	-	
GA at sampling weeks	31.16 ± 1.0	30.86 ± 0.5	-0.3	-0.7 to 0.2	0.248
GA at delivery weeks	35.16 ± 0.9	31.16 ± 1.3	-4.0	-4.5 to -3.4	<0.0001
Serum CRP level (mg/dl)	4.91 ± 0.51	7.65 ± 1.42	2.75	2.35 to 3.14	<0.0001
Cervicovaginal VDBP (µg/ml)	1.21 ± 0.64	2.46 ± 1.26	1.25	0.84 to 1.66	<0.0001

Data are mean ± SD or number (%),
95% CI = 95% confidence interval.
*Unpaired t-test unless otherwise indicated.
#Fisher's exact test.

Table 2: Receiver-operating characteristic (ROC) curve analysis for prediction of spontaneous PTL within 48 hours using serum CRP or cervicovaginal VDBP

ROC curve metric	Predictor	
	Serum CRP	Cervicovaginal VDBP
Area under the ROC curve (AUC)	0.971	0.781
Standard Error	0.027	0.075
95% Confidence interval b	0.913 to 0.994	0.684 to 0.860
z statistic	17.536	3.756
P-value (Area=0.5)	<0.0001	0.0002
Youden index J	0.907	0.592
Associated criterion (Best cut-off)	>5.7 mg/l	>2.3 µg/ml
Sensitivity	94.74	63.16
95% CI	74.0 - 99.9	38.4 - 83.7
Specificity	96	96
95% CI	88.8 - 99.2	88.8 - 99.2
+LR	23.68	15.79
95% CI	7.8 - 72.1	4.9 - 50.4
-LR	0.055	0.38
95% CI	0.008 - 0.4	0.2 - 0.7
+PV	85.7	80
95% CI	66.3 - 94.8	55.6 - 92.7
-PV	98.6	91.1
95% CI	91.4 - 99.8	85.1 - 94.9

Table 3: Comparison of receiver-operating characteristic (ROC) curves for prediction of spontaneous PTL within 48 hours using serum CRP or cervicovaginal VDBP

	ROC metric		
Predictor	AUC	SE	95% CI
Serum CRP	0.971	0.027	0.913 to 0.994
Cervicovaginal VDBP	0.781	0.075	0.684 to 0.860
Comparison of ROC curves			
Difference between areas		0.189	
Standard Error		0.068	
95% Confidence Interval		0.057 to 0.322	
z statistic		2.800	
Significance level		0.005	

Table 4: Correlations of serum CRP or cervicovaginal VDBP with each other or with GA at sampling of at delivery

		Serum CRP level	Cervicovaginal VDBP
Cervicovaginal VDBP	Pearson r	0.407**	-
	P-value	<0.001	-
GA at sampling weeks	Pearson r	-0.124	0.019
	P-value	0.232	0.853
GA at delivery weeks	Pearson r	-0.745**	-.421**
	P-value	<0.001	<0.001

DISCUSSION

The principal findings of this study are as follows; in women with PTL, the CVF VDBP level independently predicts imminent preterm delivery. This study confirms the findings of previous studies conducted in women with PTL by Liong *et al.*[14] and Hitti *et al.*[7].

In accordance with the results of the current study, the previous longitudinal study by Liong *et al.* showed that VDBP is detectable in the CVF of all pregnant women during the second half of pregnancy with no change in the CVF VDBP level between 20 and 35 weeks' gestation[14] suggesting that VDBP is a physiologic constituent of the lower genital tract fluid. In addition, VDBP has been previously identified in the AF of pregnant women[8] and on other experiment to determine the dilution ratio showed that VDBP levels in the AF without intra-amniotic infection were approximately 10 times higher than the levels in the CVF (supplementary materials)[12]. Similarly, the VDBP levels reported in pregnant maternal serum were 1000 times higher than those measured in the CVF in the current study[3].

Thus, the most likely source for VDBP present in the CVF in the absence of infection or inflammation may be transudate from the maternal serum or AF.

The present study demonstrates that in women with PTL, CVF VDBP level was independently predictive of SPTD within 48 hours. These observations are consistent with the results of a previous study by Liong *et al.* who demonstrated that the CVF expression of albumin and VDBP increased significantly in women with symptoms of PTL who delivered preterm, compared with that of those who delivered at term[13]. In asymptomatic women, elevated CVF VDBP levels have been previously reported to be associated with spontaneous term labor, PTL and the occurrence of PPROM[14]. Similarly, a recent study demonstrated that an elevated CVF VDBP level is independently predictive of SPTD at <32 weeks in asymptomatic women with cervical insufficiency or a short cervix[16]. Taken together, these observations suggest that VDBP may be implicated in the mechanisms of premature cervical remodeling and preterm birth. On the other hand, the AUC value for the ability of VDBP to predict SPTD within 48 hours was relatively small (0.71) in the present study, which indicates that the clinical usefulness of CVF VDBP is limited when used alone, and highlights the need to combine various predictors for preterm birth in light of its multifactorial and complex etiology. Therefore, further studies are warranted to explore the utility of VDBP levels in CVF in combination with other important clinical factors (e.g., fetal fibronectin level and sonographic cervical length) in the prediction of SPTD.

We expanded the observations reported by Hitti *et al.* by demonstrating that this relationship is independent of other risk factors for inflammation in PTL, such as gestational age at sampling and serum CRP[10]. Ma *et al.* found VDBP to be expressed in the trophoblasts of normal placentas throughout pregnancy[15]. Guha *et al.* have shown that IL-6 can increase the synthesis of VDBP in vitro[6]. Alternatively, the CVF VDBP level can be significantly elevated in the context of the high local production of this protein in the cells of the cervix or vagina that occurs in response to vaginal or cervical infection associated with ascending infection (e.g., bacterial vaginosis)[1].

Our study had several limitations; first, we lacked data on other potentially important tests that could contribute to the assessment of the risk of SPTD (i.e., fetal fibronectin and sonographic cervical length)[13]. Second, the current study did not include information on confounding factors for the relationship between the VDBP level and outcomes, such as recent sexual intercourse, bacterial vaginosis, vaginal microbiology, and CVF albumin level, although these variables (except for CVF albumin level) have not been previously implicated as confounders of CVF VDBP levels[13]. Third, the study was of an observational retrospective nature, which may lead to not correcting for major potential confounders despite conducting multivariate analysis. Fourth, this study was performed at a single hospital, which may limit the generalizability of our findings. Therefore, the results of this study need to be prospectively validated in a different, larger population. Fifth, the current study was limited by the fact that the samples were not randomly analyzed; thus, the significance of the findings, when used for population inference, may have an error to an unknown degree. Sixth, the fact that VDBP was assayed in stored samples after the development of the measured outcomes prevented any effect of the VDBP levels on the decision-making process regarding each case. Seventh, our study was limited by the fact that multiple testing corrections were not applied owing to the explorative nature of the study, which may lead to false-positive results. Finally, we did not obtain test results for lower genital tract microbiology, even though it may affect the CVF VDBP level according to a reported positive correlation between bacterial vaginosis and IL-1α and IL-1β levels[11]. Therefore, the current study did not clarify whether the origin of the elevated CVF VDBP levels is lower genital tract infection or intra-amniotic infection. The main strength of the study is that it is, to the best of our

knowledge, the first study to characterize the change in the CVF VDBP levels in association with SPTD in a relatively large cohort of symptomatic women, where stratification was based on the intact fetal membrane status.

CONCLUSION

In conclusion, we demonstrated that the level of VDBP in the CVF independently predicted imminent preterm delivery in women with PTL but limited when used alone, and highlights the need to combine various predictors (e.g., fetal fibronectin level and sonographic cervical length) for preterm birth in light of its multifactorial and complex etiology.

CONFLICT OF INTEREST

There are no conflicts of interests.

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