Correlation between cervical consistency index and vitamin D binding protein in prediction of preterm labour

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Abstract: Background: Prediction of preterm delivery is not an easy process and requires high obstetriccare of pregnant women at risk, or presented with threatened preterm labour. Sonographic evaluation of cervix began in 1970s, and development oftransvaginal probe a decade later improved its accuracy and diagnostic value inevaluation of cervix in pregnancy. **Objective**: to assess the diagnostic power of a CCI obtained using TVU and evaluates the correlation between it and vitamin D binding protein serum level in prediction of preterm labour. **Patients and Method**: A prospective case control study was done on 45 pregnant attending Al Zahraa University Hospital & El Khanka Hospital (30 case and 15 control) from June to December 2015. Pregnant woman with symptoms suggestive of preterm labour, single tone or twin gestations, intact amniotic membranes, past history of PTL and in the stages of pregnancy from 24 to 28 weeks gestation were included in the study. Pregnant women with major fetal anomaly, confirmed membrane rupture, cervical cerculage, abruption placenta or those with intra uterine growth restrictions were excluded from the study. Measurement of vitamin D binding protein in maternal serum by ELISA technique and TVU to detect CCIwere done to all cases. **Result:** Preterm delivery showed significantly lesser AP" diameter and CCI at 28 weeks when compared to those delivered at term, while AP diameter, AP', CCI at 24 weeks and AP at 28 weeks did not differ significantly between preterm and term deliveries. Term deliveries showed significant decrease in AP diameter, AP', and CCI at 28 weeks when compared to 24 weeks (p=0.001 for each). Cervical AP' was significantly lower when compared to cervical AP at 24 and 28 weeks (p=0.001 for both). While at preterm there was a significant decrease in AP diameter, AP', and CCI at 28 weeks when compared to 24 weeks (p<0.001 for each). Cervical AP' was significantly lower when compared to cervical AP at 24 and 28 weeks (p<0.001 for both). Regarding the VDBP, there was a significant increase at 28 weeks when compared to 24 weeks. There were no significant differences found between term and preterm groups at 24 and at 28 weeks. **Conclusion:** CCI showed the highest specificity, sensitivity, positive and negative predictive values than VDPB. This results support the use of TVS in all cases at high risk or presented with threatened preterm labour for detection of cervical parameters especially CCI, aiding in early prediction of preterm labour with confirmation by serum VDBP.
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Keywords: Preterm labour**,** cervical consistency index**,** vitamin D binding protein**,** trans vaginal ultrasound.

1. Introduction

Preterm labour is the presence of uterine contractions of sufficient frequency and intensity to affect progressive effacement and dilatation of the cervix prior to term gestation (between 20 and 37 wk). Preterm labor precedes almost half of preterm births and is the leading cause of neonatal mortality in the United States ***(ACOG 2014).***

Its incidence is about 5% to 8% in most developed and developing countries. This incidence is increasing in many countries, including developing countries, despite extensive research efforts ***(Berghella et al., 2013).***

Spontaneous preterm labor in pregnancies with intact fetal membranes represents the largest cause of preterm delivery accounting for about half of preterm births ***(Cunningham et al., 2010).***

Chronic maternal medical disorders can be associated with maternal or fetal complications necessitating iatrogenic (ie, medically indicated) or induced preterm delivery ***(Robinson et al., 2015).***

The identification of risk factors for predicting preterm birth is advantageous because it allows for the initiation of risk-specific treatment for at risk women and these risk factors may provide insights into a better understanding of the mechanisms leading to preterm birth ***(Ronna 2014).***

Different cervical parameters have been evaluated as predictors of PTB. Many parameters other than CL and presence or absence of a funnel have been studied, including funnel width, funnel length, anterior and posterior cervical width, cervical angle, cervical position (horizontal versus vertical), lower uterine segment thickness, vascularity, visibility

of chorion or amnion at internal os***(Berghella et al., 2013).***

Cervical consistency index (CCI) represents the ratio of cervical width after compression (AP'') to the width before compression (AP). The cervical width is always measured perpendicular to the longitudinal cervical axis. Thus the equation is: CCI = (AP" / AP) × 100 ***(Parra-Saavedra et al., 2011).***

A higher CCI is associated with reduced cervical elasticity, and the CCI shows inverse linear relationship with gestational age, also CCI is independent on cervical length or width of the cervical lips and this means that cervical elasticity is independent on physical dimensions ***(Al Naimi et al., 2014).***

This study was designed to assess the diagnostic power of a new cervical consistency index (CCI) obtained using trans vaginal ultrasound and evaluate the correlation between it and vitamin D binding protein serum level in prediction of PTL in order to interfere early and decrease morbidity and mortality.

2. Patients and methods

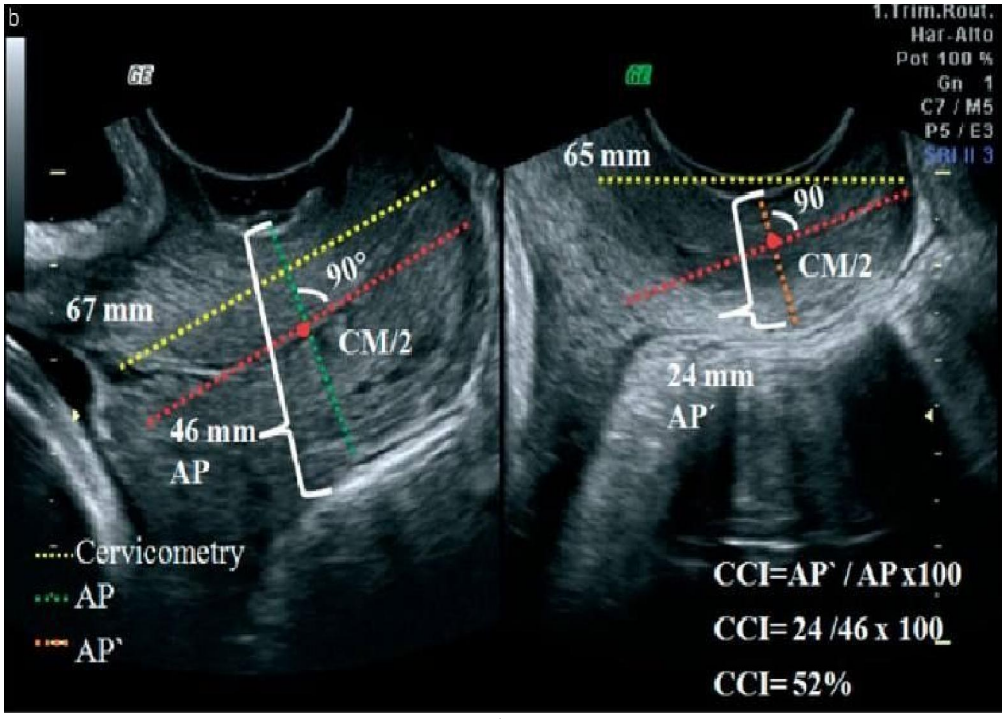
This prospective case control study was done on 45 pregnant woman attending ante natal care unit at Al zahraa University Hospital & El Khanka Hospital (30 cases and 15 controls) from June 2015 to December 2015 after informed consent. The patients enrolled in the study were the pregnant women with symptoms suggestive of PTL as uterine contraction, low back ache and vaginal discharge with singleton gestations in a stage of pregnancy from 24 to 28 weeks with intact amniotic membranes and past history of PTL in multipara. Pregnant women in the stages of pregnancy before 24 or after 28 weeks gestation or those with major fetal anomaly, confirmed membrane rupture, cervical dilatation >3 cm, cervical cerculage, vaginal bleeding, intra uterine growth restriction, preecclampsia, medically indicated preterm delivery before 35 weeks of gestationor cervical manipulation or intercourse within the previous 24 hours were excluded from the study. All studied cases were subjected to the following: A) Complete history taking including patient age, parity, gestational age, symptoms and past history of PTL. B) Complete physical and obstetric examination as fundal level and uterine contraction. C) Ultrasound examination: Abdominal U/S to detect: fetal age, viability and liqour amount and trans vaginal U/S to detect cervical consistency index. D) Laboratory investigations including Complete Blood Count (CBC), urine analysis, random blood sugar, measurement of vitamin D binding protein in maternal serum by ELISA technique. All the previous examinations were done at 24 and 28 weeks of gestation, and follow up was done to see which of these patients proceeded to PTL and which continued to term gestation.

To determine the CCI, the following steps were performed as in **fig (1a & b).** (a) 1: the cervical length is measured (in mm). 2: a line showing the cervical length is drawn on both sides of the screen (yellow lines), which is then shifted to align with the longitudinal axis of the cervix (red lines). 3: the mid-point of the cervical length is calculated (CM/2) on both sides of the screen. (b) 4: the anteroposterior (AP) diameter is measured on each side of the screen perpendicular to the longitudinal axis of the cervix and through point CM/2, from the most anterior to the most posterior lip of the cervix (white bracket). On the left side of the screen this diameter is termed AP and on the right side it is termed AP'. 5: To obtain the CCI: (CCI = (AP'/AP) × 100).

Laboratory procedure: Sample collection and storage: 2 cm of maternal blood were taken, centrifuged to separate the serum, and then re-frigenation of the samples at -20c, until all samples were collected, then the assay was done using Human Vitamin D-binding Protein (DBP) ELISA Kit, Catalog Number, CSB-E11859h-China, produced by CUSABIO. This assay employs the competitive inhibition enzyme immunoassay technique; the micro titer plate provided in this kit has been pre-coated with DBP. Standards or samples are added to the appropriate micro titer plate wells with Horseradish Per oxidase (HRP) conjugated antibody preparation specific for DBP. The competitive inhibition reaction is launched between with precoated DBP and DBP in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of DBP in the samples. The color development is stopped and the intensity of the color is measured.

The statistical analysis of data was done by using Excel program (Microsoft Office, 2013) and IBM SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Kolmogorov-Smirnov test was done to test the normality of data distribution. Quantitative data were presented as mean, standard deviation, median and range. For comparison between two groups; student t-test, and Mann-Whitney test (for non-parametric data) were used. Qualitative data were presented as number and percentage. Chi square and Fisher exact tests were used to compare categorical groups. Diagnostic performance was determined by constructing a ''receiver-operating characteristic'' (ROC) curve and calculating the area under the ROC (AUROC) curve. From these curves, sensitivities, specificities and the best cut-off values were established, which were the values that maximized the sum of the sensitivity and specificity to identify patient status. Diagnostic validity was estimated using sensitivity, specificity, positive (PPV),

negative (NPV) predictive values and accuracy. Correlation coefficient was used to examine the correlation between parameters. Logistic regression was used for prediction of risk factors associated with preterm labor.



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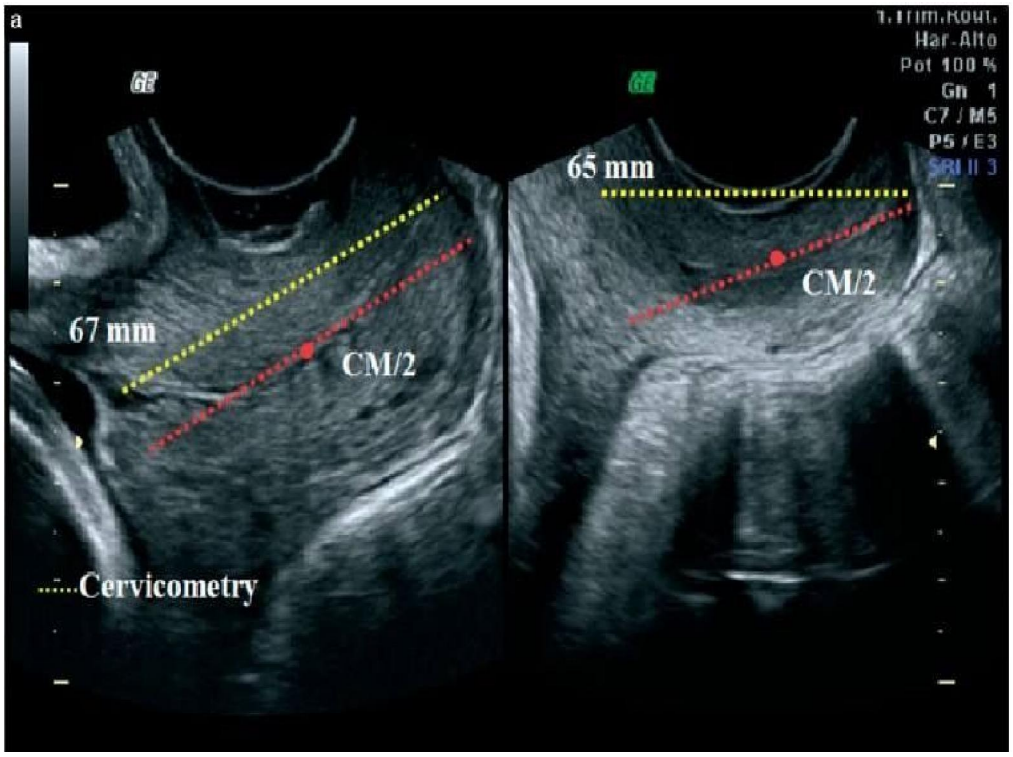

B

Fig (1a & b): Ultrasound images with annotation illustrating the assessment of cervical consistency index (CCI).

3. Results

The mean age of studied cases who were subjected to preterm labor was 26.3 years (SD=4.5) while the mean age of control group who delivered at term was 24.7 years (SD=5.5). The median number of parity of preterm group was 1 as the term group. The preterm group ranged from nullipara to para 4, while the term group ranged from nullipara to para 5. Median gestational age at delivery for those delivered by preterm labor was 35 weeks, while for term labor it was 39 weeks (p<0.001).

Females who delivered at preterm showed significantly lesser AP" diameter and CCI at 28 weeks when compared to those delivered at term. On the other hand, AP diameter, AP', CCI at 24 weeks and AP at 28 weeks did not differ significantly between both groups Table (1). It was found that females who delivered at term showed significant decrease in AP diameter, AP', and CCI at 28 weeks when compared to 24 weeks (p=0.001 for each). Cervical AP' was significantly lower when compared to cervical AP at 24 and 28 weeks (p=0.001 for both). While at preterm there was a significant decrease in AP diameter, AP', and CCI at 28 weeks when compared to 24 weeks (p<0.001 for each). Cervical AP' was significantly lower when compared to cervical AP at 24 and 28 weeks (p<0.001 for both).

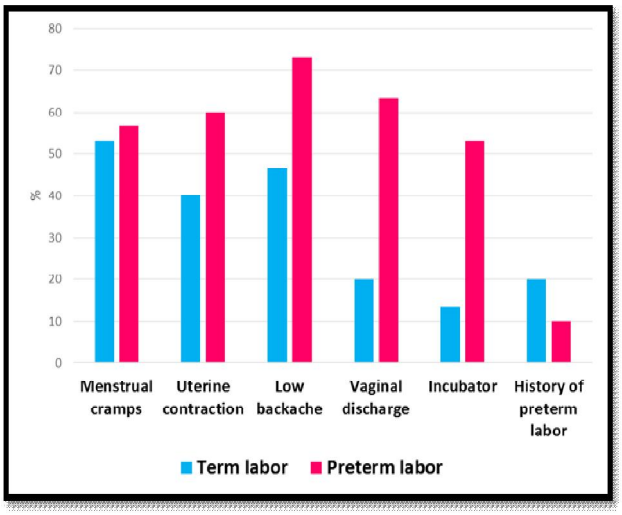

Fig (2): Clinical manifestations and history of PTL for all studied pregnant females

Table (1): Sonographic cervical measurements of all studied pregnant females

	Term labor N=15 \ 45		Preterm labor N=30 \ 45		*p*
	Median	Range	Median	Range	
24 W cervical (AP) at	32.5	27.8- 39.7	34.85	23.3-44.6	0.198
24W cervical (AP) aftercompression	26.6	20.6-32.2	25.95	16.5- 35.6	0.682
24 weeks CCI	79.6	66.1- 89.3	74.15	48.5- 92.6	0.243
Cervical (AP) at 28 W	29.7	24.7- 34.6	28.15	22.6-37.5	0.555
28W cervical (AP) aftercompression	21.2	15.8-25.6	15.3	9.5-21.8	<0.001
28 weeks CCI	70.4	62.7-82.1	54.45	29.1-71.7	<0.001

Statistical analysis of the given data showed that the Receiver Operating Characteristic (ROC) curve of CCI was conducted at24 and 28 weeks for prediction of preterm labor. CCI at 28 weeks showedexcellent Area under the Curve (AUC) (AUC=0.982, p<0.001) at cut offvalue of < 62. While CCI at 24 weeks showed poor AUC (AUC=0.608, p=243), at cutoff value of <73.8. Performance characteristics are shown in table (2).

Table (2): Area under the curve and performance characteristics of CCI at 24 and 28 weeks for prediction of preterm labor in all studied groups

CCI	Cut off	AUC	*p*	95% CI	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Accuracy(%)
at 24 weeks	<73.8	0.608	0.243	0.439-0.776	50	80	83.3	44.4	60
at 28 weeks	<62	0.982	<0.001	0.946-1	96.7	100	100	93.8	97.8

Table (3): Comparison between VDBP at 24 and 28 weeks in allstudied groups

VDBP (ug/ml)	Term labor (N=15)		Preterm labor (N=30)		P[1]
	Median	Range	Median	Range	
at 24 weeks	508.53	430.52-580.13	522.92	430.64-776.69	0.819
at 28 weeks	599.82	507.54-799.76	622.89	461.40-957	0.341
P[2]	0.001		<0.001		

Regarding the VDBP, there was a significant increase at 28 weeks when compared to 24 weeks. There were no significant differences found between term and preterm groups at 24 and at 28 weeks.

ROC curve of VDBP was conducted at 24 and 28 weeks forprediction of preterm labour. AUCs of VDBP at 24 and 28 weeksfailed to discriminate between preterm and term labour. Performance characteristics are shown in table (4).

Table (4): Area under the curve and performance characteristics of VDBP at 24 and 28 weeks for prediction of preterm labor in all studied groups

VDBP	Cutoff	AUC	*p*	95%CI	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Accuracy(%)
at 24 w	>588.1	0.521	0.819	0.350-0.692	20	100	100	38.5	46.7
at 28 w	>688.3	0.588	0.342	0.423-0.752	40	93.3	92.3	43.8	57.8

Regression analysis was conducted for prediction of preterm labor using age, parity, history of preterm labor, CCI at 24, 28 weeks, VDBP at 24 and 28 weeks as co-variantes. CCI at 28 weeks was considered a predictor of preterm labor. It was found that the higher the CCI at 28 weeks, the lower risk to develop preterm labor. Other co-variants were not considered as predictors for preterm labor in the current study.

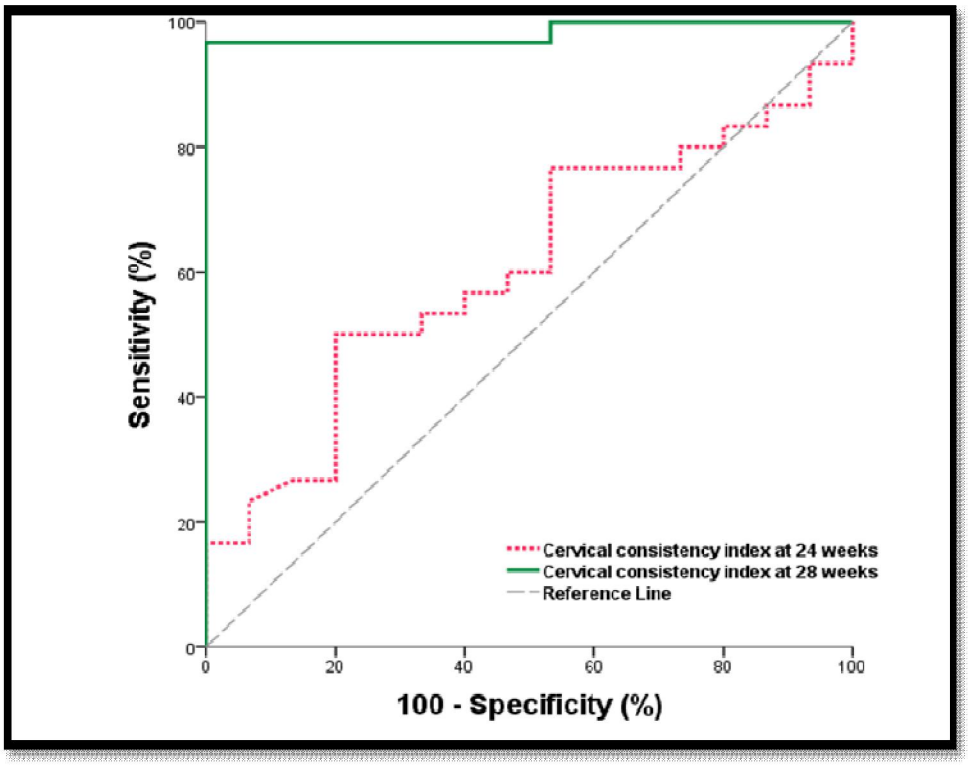

Fig (3): ROC curve of CCI at 24 and 28 weeks for prediction of preterm labor.

Table (5): Regression analysis for prediction of preterm labor

	P	OR 95%	CI
Age (year)	0.305	1.073 0.938-	1.227
Parity	0.526	0.861 0.542-	1.368
History of preterm labor	0.361	0.444 0.078-	2.529
CCI at 24 weeks	0.221	0.955 0.887-	1.028
CCI at 28 weeks	0.004	0.639 0.473-	0.864
VDBP at 24 weeks (ug/mL)	0.444	1.004 0.994-	1.013
VDBP at 28 (ug/mL)	0.127	1.004 0.999-	1.010

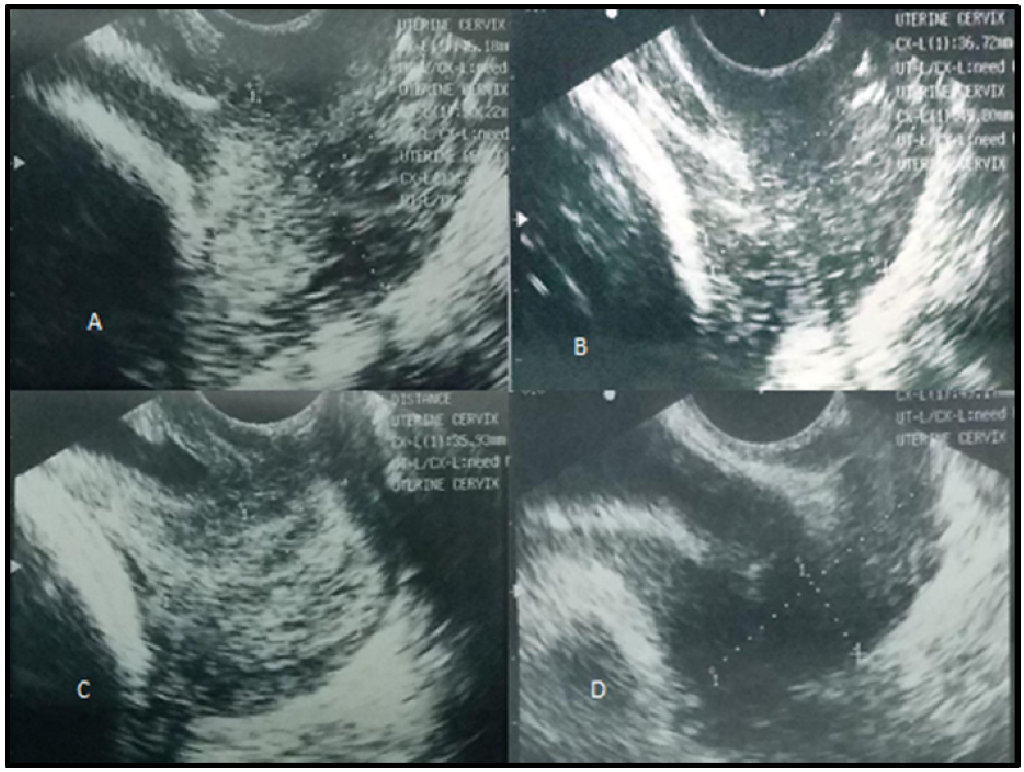

Fig (4): a-TVU measuring (AP) of the cervix before compression at 24w = 39.68 mm. **b-** (AP') after compression at 24w = 30.13 mm. **c-** (AP) before compression at 28 w= 28.13 mm. **d-** (AP') after compression at 28w = 17.25 mm. **CCI** at 24 w 75.9 = **CCI** at 28w = 61.32This case of threatened preterm labour was presented withpreterm labour pains and delivered pre term at 34 weeks' gestation.

Fig (5): a- TVU measuring (AP) of the cervix before compression at 24w = 33.96mm. **b-** (AP') after compression at 24w = 29.55 mm. **c-** (AP) before compression at 28 w= 29.69mm. **d-** (AP') after compression at 28w = 24.38mm. **CCI** at 24 w 87.01= **CCI** at 28w = 82.11. This case of threatened preterm labour was presented withpreterm labour pains and delivered pre term at 39 weeks' gestation.

4. Discussion

Early detection of pregnant women who will deliver before term has beensought as a chance to reduce the occurrence of prematurity related peri natalmorbidity and mortality ***(ACOG 2013).*** Preterm birth is associated with a range of adverse outcomes for the baby. These include increased rates of perinatal death, neonatal morbidity (includingrespiratory distress syndrome, intra ventricular haemorrhage and necrotizingenterocolitis) and long term compromise. The main areas affected in the long termare the neurological system (which can result in cerebral palsy and lowereducational attainment) and respiratory system (for example, broncho pulmonarydysplasia**)** ***(NICE 2015).***

The objective of this study is to compare the value of measuring cervicalconsistency index 'CCI' by trans vaginal ultrasound versus serum vitamin Dbinding protein 'VDBP' in prediction of preterm labour.

In this study, 45 patients with preterm labour symptoms were assessed at 24 and 28 weeks of gestation by TVUS, calculate CCI and serum VDBP was measured.

As regard sonographic cervical measurements females who delivered preterm showed significantly lesser CCIat 28 weeks when compared to those delivered at term. Also, females who delivered at term showed significant decrease in APdiameter, AP', and CCI at 28 weeks with more decrease in AP' when compared toAP diameter at 24 and 28 weeks ($p=0.001$ for both). Females delivered preterm showed significant decrease in AP diameter, AP', and CCI at 28 weeks when compared to 24 weeks ($p<0.001$ for each). Cervical AP' was significantly lower when compared to cervical AP at 24 and28 weeks ($p<0.001$ for both). On the other hand, AP diameter, AP', CCI at 24 weeks and AP at 28 weeks didnot differ significantly between both groups.

This was explained by the study carried out by ***Parra-Saavedra et al. (2011),*** as theyfound that the cervix of pregnant women loses an average of 1.2% of consistencyper week of pregnancy and shows a clear inverse linear relationship between CCIand gestational age (GA), with the following equation: CCI (in %) = 89.8 − 1.35 × (GA in weeks); r2 =0.66, $P < 0.001$. There weresignificant differences in CCI between the three trimesters ($P < 0.0001$), with anaverage CCI of 59.4% for the second trimester, near our cut off point which is 62. Our study also found the CCI shows a clear inverse linear relationship withgestational age, and it was also lower in pregnancies that went on to deliverpreterm and this matches with the results of the study carried out by ***Parra-Saavedra et al. (2011).***

In this study, mathematically, CCI at 28 weeks showed excellent Area under the Curve (AUC) (AUC=0.982, $p<0.001$) at cut off value of < 62 with 100% specificity, 96.7% sensitivity, 100% PPV, 93.8% NPV and 97.8% accuracy, While CCI at 24 weeks showed poor AUC (AUC=0.608, p=243), at cut off value of <73.8 with 80% specificity, 50% sensitivity, 83.3% PPV, 44.4% NPV and 60% accuracy. On multivariable logistic regression analysis in the study of ***Parra – Saavedra et al. (2011)***, the only variable that showed statistical significance in the prediction of PTB was CCI percentiles according to gestational age and this coincides with the results of our study, as CCI at 28 weeks was considered a predictor of preterm labor, the higher the CCI at 28 weeks, the lower the risk to develop preterm labor. This study also proved that there is positive correlation between CCI measured by TVUS and preterm labour as females who delivered preterm showed significantly lesser CCI at 28 weeks when compared to those delivered at term.

On the other hand, we have found that VDBP showed significant increase at 28 weeks when compared to 24 weeks and this matches with the study of ***Bouillon et al., (2015)*** as they have found a plateau at about 20 weeks, and a further increase at 28-34 weeks. In their study, **Bouillon *et al.,(2011)*** have measured the serum concentration of VDBP of 110 pregnant patients: a rise starting at about 10 weeks of gestation, a plateau at about 20 weeks, a further increase at 28-34 weeks and a small decrease during the last weeks of pregnancy was observed.

This study showed that there are no significant differences of serum VDBP were found between term and preterm groups at 24 and at 28 weeks and AUCs of VDBP at 24 and 28 weeks failed to discriminate between preterm and term labor.

Another novel study carried out by ***Liong et al., (2013)*** characterizing the temporal changes of VDBP in the CVF in association with pregnancy and spontaneous labour, In the final four weeks of a term singleton pregnancy, they found that the VDBP concentration in the CVF was significantly elevated at 40–41 weeks' compared to 36–37 weeks' gestation. In addition they have found that beyond 37 weeks' gestation, the CVF concentration of VDBP significantly increases independent of gestation, suggesting that VDBP increases in association with approaching labour. Their preterm data also supports this finding as a significantly increased concentration of VDBP was found in samples collected from women 28 days from labour who spontaneously delivered preterm, compared to women who delivered at term. This is an interesting observation and may indicate that women subjected for a preterm birth have a higher

'baseline' expression of VDBP in the CVF. Also, VDBP was able to predict spontaneous labour onset with high positive and negative predictive values up to 14 days from sampling.

Conclusion

Cervical changes can be very important in predicting preterm birth in preclinical stage, thus considering this issue as part of perinatal medicine and start cervical assessment even in the first trimester. CCI by TVUS should be done as a routine test in prediction of preterm labour. Our results showed that CCI measurement sensitivity, specificity, PPV, NPVand accuracy (96.7%, 100%, 100%, 93.8%, 97.8% respectively) at 28 weeks'gestation were more predictive for preterm labour than serum VDBP sensitivity, specificity, PPV, NPV and accuracy which were (40%, 93.3%, 92.3%, 43.8%,57.8% respectively) at 28 weeks' gestation. CCI is a cheap, easy, non-invasive tool with high accuracy in prediction ofpreterm labour. This initial study opens the possibility to do research on a new way ofassessing changes in the cervix that occur during pregnancy, soadditional larger and prospective studies are needed for further evaluation of this unique and important parameter in cervicalassessment and its comparison with other old parameters.

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