Original Article

Evaluation of transvaginal ultrasonography, power Doppler indices, electromyography and serum protein 53 level in prediction for preterm labour

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ABSTRACT

Objective: The objective of this study is to evaluate the role of cervical length measurement by transvaginal ultrasonography, power Doppler of the uteroplacental circulation, electromyography and serum protein 53 level (p53) in prediction for preterm labour (PTL).

Study Design : A prospective observational study.

Patients and Methods: The study was conducted at the outpatient clinic of El-Minia University Hospital, Egypt during the period from January 2012 to December 2014. The study included a total of 100 pregnant women at high risk for preterm labor. All included cases were subjected to transvaginal cervical sonography, power Doppler velocimetry and uterine electromyography measurements (EMG), also, serum protein 53 level was determined.

Results: The included subjects were classified into two groups according to gestational age at labor: Group (I): patients delivered before < 35 weeks (n=42) and group (II): patients delivered ≥ 35 weeks (n=58). Group (I) had significantly lower cervical length compared to group II (2.86 ± 0.81vs. 3.23 ± 0.67 cm) (*P=0.013*). There was a significant difference between groups as regards EMG (*P = 0.006*). No statistically differences were found between groups as regards Doppler indices (both resistance and pulsatility index). Group (I) had significantly higher maternal serum concentration of P53 compared to group II (473.8 ± 146.5 vs. 404.6 ± 164.7 pg/ml), (*P≤ 0.01*). Serum P 53 level had the higher sensitivity for prediction of PTL (78.6%), however, power Doppler indices had the lowest sensitivity (RI = 23.8% and PI =19.1%), cervical length had sensitivity of 33.3% however, EMG positive parameters had sensitivity of 40.5 % and specificity of 100.0%.

Conclusion: From the studied tools for prediction of preterm delivery, different results were obtained, but serum P53 level was the most sensitive and the best option for prediction of preterm delivery, while, power Doppler indices are the least sensitive. However, EMG positive parameters are the most specific for prediction of PTL.

Key Words: Electromyography, preterm labour, prediction, power Doppler indices, protein 53, transvaginal ultrasonography

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INTRODUCTION

Preterm delivery remains a worldwide problem, it is defined as delivery at less than 37 weeks of gestation and it is associated with obvious increase in perinatal morbidity (low birth weight, growth debilitation and damage of the nervous system) and increased risk of developing respiratory distress syndrome, intraventricular hemorrhage, bronchopulmonary dysplasia, sepsis, necrotizing enterocolitis, patent ductus arteriosus and further, it is the second leading cause of perinatal mortality (nearly 30% of all neonatal deaths)[1]. Globally, its incidence ranges from 5:15% (about of 15 million babies annually), making this disorder has overall clinical, social, and economic burden[2]. The major subcategories of preterm birth are spontaneous preterm labor, delivery for maternal or fetal indications and preterm premature rupture of membranes (PPROM), multifactorial etiology of spontaneous preterm labor causes the difficulty to identify and consequently prevent it[3].

Nowadays, prediction and prevention of preterm delivery stay of the great challenges. The etiology of preterm delivery and its exact mechanism is largely unclear, it is complex and influenced by many factors such as genetics, gestational age, history of PTL, psychological and pregnancy complications, placenta previa, cervical length measurements and obesity[1]. Many different methods were used for prediction of preterm birth ; of these, cervical morphology and biometry, biomarkers such as fetal fibronectin "FFN", salivary estriol, cervicovaginal intracellular adhesion molecule-1 "ICAM-1", maternal

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serum alpha fetoprotein "MS-AFP", cervicovaginal beta-human chorionic gonadotropin "β-hCG", phosphorylated insulin-like growth factor binding protein-1 "phIGFBP-1"[4], in addition to hospital tocodynamometry for monitoring uterine contractions to evaluate preterm labor[5]. Recently, power Doppler indices[6], electromyography[7] and assessment of serum protein 53 (P53) level[8] have been introduced in clinical practice to offer some advantages and seeking to improve the sensitivity and specificity in prediction of preterm delivery over the traditional used methods and consequently this can help to avoid substantial economic costs associated with unnecessary hospitalization, the maternal risks associated with tocolytics, and the potential fetal risks associated with steroids[9]. The objective of this study is to evaluate the role of cervical length measurement by transvaginal ultrasonography, power Doppler of the uteroplacental circulation, electromyography and serum protein 53 level in prediction for preterm labour.

PATIENTS AND METHODS

This is a prospective observational study included a total of 100 pregnant women at high risk for preterm labor who were selected from the attendants of obstetrics outpatient clinic of El-Minia University Hospital, Egypt during the period from January 2012 to December 2014. Prior to the procedure, all the benefits, risks and possible complications of the procedure were clearly explained to the patient and an informed consent was taken from each patient. Women were included if they had gestational age between 24 and 28 weeks, single viable fetus, absent major congenital fetal malformation, normal fetal heart rate, intact membrane, normally situated placenta without separation and no medical disorder or obstetric complication. We exclude cases with history of unexplained vaginal bleeding, PROM, Polyhydraminos, acute febrile disease or cervical conization or cauterization, abnormal uterine cavity in addition to cervical diltation >2 cm. All included cases were subjected to thorough history taking, general and physical examinations ; also, full obstetric examination was done including routine ultrasound scanning to exclude pregnancy abnormalities such as IUGR, oligohydramnios or multiple gestation. In addition, sterile speculum examination was done to detect cervical diltation, consistency, effacement and position of the cervix. Routine laboratory investigations were done for all included cases and also serum protein 53 (P53) level was measured by ELISA method using Biotinylated standard kits by Bio-Rad.

Transvaginal cervical sonography was conducted with commercially available two-dimensional (2D) ultrasound system (VOLUSON S8 Gereral electric) equipped with endovaginal transducers with frequency ranges of 5-10 MHz, also equipped with color, power and pulsed Doppler capabilities. Power Doppler velocimetry of the uterine artery and umbilical artery was evaluated at 24-28 weeks of gestation by abdominal ultrasound using (VOLUSON S8 Gereral electric). Doppler indices generated automatically from the machine, the pulsatility index (PI), resistance ndex (RI) and S/D ratio are recorded. Resistance index (RI) was calculated from the uterine artery, abnormal RI was > 0.58 and very abnormal RI was ≥ 0.7. Uterine Electromyography (EMG) measurements were done within one day from the admission of the patients to the hospital. Non-invasive trans-abdominal surface electrodes were used for recording EMG activity, uterine EMG was measured for 30 minutes using a custom-built uterine EMG patient-monitoring system. To obtain a final band-pass of 0.34 to 1.00 Hz, Analog EMG signals were digitally filtered in order to exclude most components of respiration and or motion in addition to cardiac signals from the analysis and data was sampled at 100 Hz (so as to increase the resolution of PS analysis later). All women had been followed up carefully at 2-4 weeks intervals till time of delivery and recording the time of labour, the studied sample was then classified into two groups according to gestational age at labor as follow: group (I): patients delivered before <35 weeks and group (II) : patients delivered after >35 weeks.

Statistical analyses were performed using the statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA, version 16). Shapiro–Wilk test was used for testing normality of the data for all continuous variables. Categorical data was presented in the form of No. (%) ; however, continuous data was established as mean ± standard deviation (SD). Comparisons between groups were done by Chi-square test for categorical data and independent sample T-test or Mann–Whitney U-test for continuous data. $P \leq 0.05$ was considered statistically significant and $P \leq 0.01$ is considered as highly significant. Evaluation of predictive power was based on sensitivity, specificity, positive and negative predictive values, efficacy and receiver operating characteristic curve (ROC curve).

RESULTS

This study included a total of 100 cases ranging from 17:38 years old with gestational age ranged from 24:28 weeks. Of them, 42 cases delivered before <35 weeks (group I) and 58 delivered after >35 weeks (group II). The results showed that there was no difference between the two groups regarding their gravidity, parity, BMI and pervious PTL. However, group II had significantly higher age ($P \leq 0.01$), (Table 1).

Table 1 : Baseline and clinical characteristics between groups

Variable	Gestational age at labor Group (I) Delivery at < 35 wks. (n=42)	Group (II) Delivery at ≥ 35 wks. (n=58)	P. value (Sig.)
Age (years)	27.4 ± 5.6 (17-38)	30.5 ± 5.9 (17-38)	<0.01**
Gravidity	3.71 ± 1.99 (1-10)	3.87 ± 1.83 (1-10)	0.67NS
Parity	2.09 ± 1.55 (0-6)	2.41 ± 1.57 (0-8)	0.32NS
BMI (Kg\m2)	21.83 ± 2.61 (17-31)	21.63 ± 2.71 (16-33)	0.72NS
Pervious PTL (+ve)	24 (57.1%)	33 (56.9%)	0.98NS
Pervious 2nd TA (+ve)	5 (11.9%)	13 (22.4%)	0.17NS

Continuous data was presented as mean ± SD (range), categorical data was presented as No. (%)
NS Not significant** Significant (*P ≤ 0.01*)

There was significant difference between groups in cervical length, group I had significantly lower cervical length compared to group II (2.86 ± 0.81 vs. 3.23 ± 0.67 cm), (*P=0.013*) and also had significantly lower number of cases who had cervical length ≥2.5 cm (*P=0.025*). Regarding uterine electromyography, the total number of patients who had positive EMG were 17 cases (40.5%) in group of women who delivered at < 35 weeks (group I) compared to no cases in group of women who delivered at ≥35 wks. (group II) and this difference was highly significant (*P≤ 0.01*). No statistically significant differences were found between groups as regards Doppler indices (both resistance and pulsatility index), almost, cases who had high resistance and pulsatility index were the same in the two groups. There was a highly significant difference in the mean maternal serum concentration of P53 between group I (473.8 ± 146.5 pg/ml) and group II (404.6 ± 164.7 pg/ml) (*P≤ 0.01*), in the same trend, number of cases who had P53 concentration ≥ 370 (pg/ml) in group (I) were higher than those in group (II), (33 cases (78.6%) vs. 27 cases (46.6%), *P≤ 0.01* (Table 2).

Table 2: Transvagianl ultrasonographic parameters, uterine electromyography, Doppler indices and serum protein 53 between groups

Variable	Groups Group (I) Delivery at < 35 wks. (n=42)	Group (II) Delivery at ≥ 35 wks. (n=58)	P. value (Sig.)
Cervical length (cm)			
Mean ± SD (range)	2.86 ± 0.81 (1.5-4.5)	3.23 ± 0.67 (2.0-4.0)	0.013*
< 2.5	14 (33.3%)	8 (13.8%)	0.025*
≥ 2.5	28 (66.7%)	50 (86.2%)	
Uterine Electromyography (EMG)			
Negative	25 (59.5%)	58 (100%)	<0.01**
Positive	17 (40.5%)	0	
Doppler indices			
Resistance index (RI) Low	32 (76.2%)	48 (82.8%)	0.42NS
High	10 (23.8%)	10 (17.2%)	
Pulsatility index (PI) Low	34 (81%)	49 (84.5%)	0.64NS
High	8 (19%)	9 (15.5%)	
Serum protein 53 (pg./ml)			
Mean ± SD (range)	473.8 ± 146.5 (79-873)	404.6 ± 164.7 (100-966)	0.03*
< 370	9 (21.4%)	31 (53.4%)	<0.01**
≥ 370	33 (78.6%)	27 (46.6%)	

Continuous data was presented as mean ± SD (range), categorical data was presented as No. (%).
RI: resistance index PI: Pulsatility index.
NS Not significant * Significant (*P≤ 0.05*)** Significant (*P≤ 0.01*)

Table 3 shows the sensitivity, specificity, PPV and NPV, accuracy of cervical length, EMG, Doppler indices and serum P53 level in prediction of preterm delivery at <35 weeks gestational age, serum P53 level is the most sensitive for prediction of PTL (sensitivity = 78.6%), however, power Doppler indices of the uterine artery are the least sensitive for prediction of PTL. EMG positive parameters are the most specific for prediction of PTL (specificity = 100.0%), followed by cervical length (<2.5cm) with specificity of 86.2% and the lowest specificity was for serum P53 (specificity = 53.5%) (Fig. 1).

Table 3: The diagnostic indices and predictive values of cervical length, EMG, Doppler indices, serum concentration of P53 used for prediction of preterm delivery at < 35 weeks gestational age.

Parameters	+ve	-ve	AUC	*P. value*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	RR	*P. value*
Cerv. length (<2.5cm)	14	8	0.598	0.02*	33.3	86.2	63.6	64.1	64%	2.42	0.03*
EMG (+ve)	17	0	0.702	<0.01**	40.5	100	100	69.9	75%	48.02	<0.01**
RI (high)	10	10	0.533	0.43NS	23.8	82.8	50	60	58%	1.38	0.41NS
PI (high)	8	9	0.518	0.65NS	19.1	84.5	47.1	59	57%	1.22	0.64NS
P53 (≥370)	33	27	0.660	<0.01**	78.6	53.5	55	77.5	64%	1.69	<0.01**

AUC: area under the curve PPV: positive predictive value NPV: negative predictive value RR: relative risk.

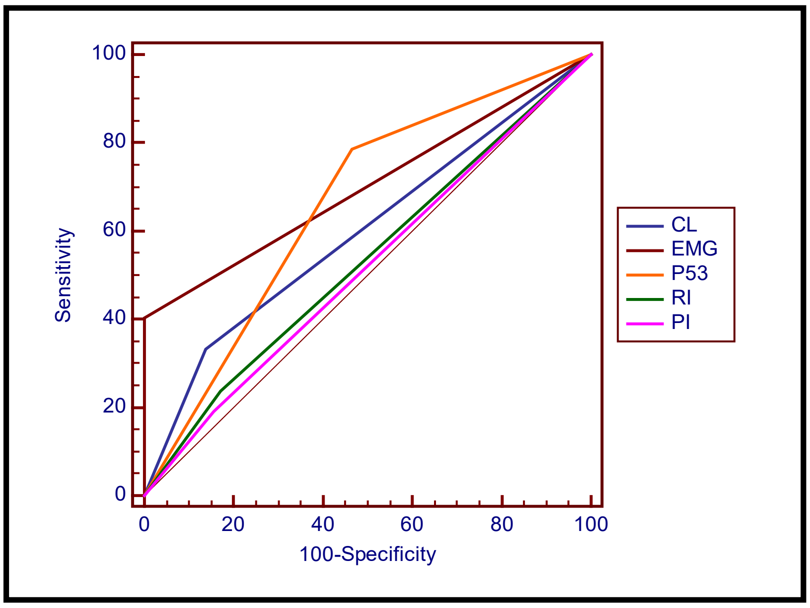

Fig. 1: ROC curve of cervical length, EMG, serum P53 concentration and Doppler indices used in the study for prediction of preterm delivery at < 35 weeks gestational age

DISCUSSION

Despite the recent advances in antenatal care, preterm delivery has remained a major cause of perinatal morbidity and mortality, furthermore, it is associated with a high incidence of severe neurological deficits and developmental disabilities[10]. The exact mechanism of preterm labor is largely unknown , but it is believed to include many factors including genetics and environmental ones[1]. Several different approaches have been proved to be clinically useful screening methods for the early prediction of preterm birth, but of these, some methods have very low sensitivity and PPV such as tocodynamometry and others have several major drawbacks. So, there is still a need to develop noninvasive and safer diagnostic method that can predict preterm labour.

In the present study, 42 cases of the total number of the studied cases developed PTL before 35 weeks ; of these, 15 cases (35.8 %) had history of previous PTL, 15 cases (35.8 %) had symptoms suggesting threatened PTL, 5 cases (12.0%) had a previous history of 2nd trimester abortion and 7 cases (16.6%) had both history of previous PTL and symptoms suggesting threatened PTL. There were no significant differences between groups in maternal age, gravidity, parity, gestational age, BMI and history of previous of PTL or previous 2nd trimester abortion, this indicates that patients came from the same population and the two groups are randomly selected and this is important to get accurate results from the comparison between groups. The rate of preterm delivery in our study was 42.0%, this rate is similar to that of Joel *et al.*[11] who reported a prevalence rate of preterm delivery of 42%, however, it is higher than that of Ehsanipoor *et al.*[12] who reported of preterm delivery rate of 19.4%.

The present study has shown that the risk of preterm delivery was high among women whose cervical length less than 25 mm ; however, also, we found that there is significant difference between groups as regards the transvagianl ultrasonographic parameters (*P. = 0.025*). In the study, 22 cases have short cervix (cervical length <2.5 cm), 14 cases of them delivered at <35 weeks (group I) ; while 8 cases delivered at ≥35-week gestational age (group II). For prediction of preterm delivery, cervical length <2.5 cm had a sensitivity, specificity, PPV and NPV of 33.3%, 68.2%, 63.2%, and 64.1%, respectively. These results are in agreement with a similar prospective study conducted by Iams *et al.*[13] performed on women with a singleton pregnancy at 24 weeks of gestation (n= 2915), they determined the predictive values of sonographic assessment of cervical length for prediction of spontaneous PTL and found that at 24 weeks, a cervical length of ≤ 25 mm had a sensitivity of 37%, specificity of 92%, PPV of 18%, and NPV of 97% in predicting spontaneous preterm birth at <35 weeks' gestation. They added that the relative risk of preterm birth before 35 weeks of gestation was about six-fold higher (95% CI: 3.84-9.97) among women whose cervical length was less than 25 mm. Similarly, Adhikari *et al.*[14] determined the association between cervical length and gestational age at delivery when measured during aroutine 2nd trimester ultrasonographic examination, they found that there is a statistically significant association between cervical length and gestational age at delivery. Furthermore, They added that cervical length <2.65 cm had a sensitivity, specificity, PPV, and NPV of 50%, 85.5%, 23.1% and 95.2%, respectively, in the prediction of the risk of early preterm birth. However, Deplange *et al.*[15] reported that the sensitivity, specificity, PPV and NPV of cervical length ≤ 20 mm were 75, 52, 21 and 92% for delivery before 34 weeks. Additionally, Paternoster *et al.*[16] found that 26 mm was the best cut-off value for cervical length in terms of predicting preterm delivery with a sensitivity of 86.4%, specificity of 71.9%, PPV of 34.5% and NPV of 96.8%.

Regarding Doppler indices, the present study has shown that there is no significant difference in resistance and pulsatility indexes of the uterine artery between group (I) vs. group (II). To predict delivery <35weeks, resistance index of the uterine artery (≥ 0.58) had a sensitivity, specificity, PPV and NPV of 23.8%, 82.8%, 50% and 60%, respectively however, pulsatility index of the uterine artery (≥ 1.04) had a sensitivity, specificity, PPV and NPV of 19.1%, 84.5%, 47.1% and 59%, respectively. These findings are in agreement with those of Cobian-Sanchez *et al.*[17] who reported that the mean uterine artery RI of cases who delivered before 34 weeks was not significantly different from that in 5472 patients who delivered at term. Also, Soares *et al.*[18] found that the mean uterine artery RI in cases with spontaneous preterm labor was not significantly different from that in 2417 pregnancies delivered at term. In contrast, Misra *et al.*[19] found that for women who delivered preterm, RI was consistently larger across all gestational ages in a longitudinal study of 523 gravidas. Also, they found that the hazard ratio for preterm delivery was statistically significant for RI (HR = 2.26, 95% CI: 1.65, 3.11). They concluded that pregnancies with higher RI through gestation are more likely to be affected by preterm delivery, suggesting that disordered placentation resulting in compromised placental blood flow may be an important pathway to preterm delivery. Also, Fonseca *et al.*[20] found that pulsatility index measured in singleton pregnancies at 22-24 weeks of gestation was significantly higher in 237 women who had a spontaneous delivery before 33 weeks than in 31,633 women delivering at or after 33 weeks.

The results of the present study has shown that the risk of preterm delivery at <35 weeks was high among women with positive EMG parameters, also, we found that there is significant difference among women delivered at < 35 weeks vs. ≥ 35 week gestational age as regards the EMG (*P. = 0.006*). In the study, 17 cases had positive EMG parameters delivered at <35 weeks ; while all cases delivered at ≥35 week gestational age had negative EMG parameters, the EMG study had sensitivity, specificity, PPV and NPV of 40.5%, 100%, 100% and 69.9%, respectively. These findings are in accordance with a similar prospective study done by Lucovnic *et al.*[7] performed on 88 women with a singleton pregnancy, they determined the predictive values of EMG for prediction of spontaneous PTL and found that positive EMG had a sensitivity, specificity, PPV and NPV of 70%, 100%, 100% and 90%, respectively for prediction of PTL within one week.

In the present study, there is no significant difference in symptoms suggesting threatened PTL among women delivered at < 35 weeks vs. ≥ 35 week gestational age. The total number that have symptoms suggesting PTL in our study was 52 cases, only 17 cases of them have positive EMG parameters and these cases were delivered within one week from the study, this means that not all cases with symptoms suggesting threatened PTL are in need for hospital admission.

The present results revealed that the risk of preterm delivery was high among women with high serum level of P53 (a cut off value of 370 pg/ml), also, we found that there is significant difference among women delivered at < 35 weeks (group I) vs. ≥ 35 week gestational age (group II) as regards the maternal serum concentration of P53 (*P.= 0.001*). We found that serum level of P53 (cut off value more than 370 pg/ml) had a sensitivity, specificity, PPV and NPV of 78.6%, 53.5%, 55% and 77.5%, respectively for prediction of PTL. Indeed, very few data is available regarding the relation between P53 and preterm delivery, but, in line with our findings, Hirota *et al.*[8] found that uterine deletion of P53 in mice increased the incidence of preterm birth. They added a further generated evidence to suggest that deletion of uterine P53 induces preterm birth through a COX2/PGF synthase/PGF(2alpha) pathway, these observations confirm the new critical role of uterine p53 in parturition. In addition, They stated that mice with accelerated decidual senescence, initiated by gene disruption of P53 in the uterus, exhibit spontaneous PTB and this PTB occurs in spite of high maternal serum progesterone concentrations, analogous to human pregnancy, but unlike several other mouse models of PTB. Furthermore, Cha *et al.* [21] reported that mice with a uterine-specific P53 conditional knockout exhibit enhanced sensitivity to environmental triggers of preterm birth. Decidual senescence has also been identified in human preterm birth, suggesting that senescence pathways such as mammalian target of rapamycin complex 1 (mTORC1) may be promising targets for intervention.

CONCLUSION

In conclusion, in comparing the diagnostic and predictive values of ultrasonographic parameters (cervical length), Doppler indices, EMG and serum P53 level used in this study for prediction of preterm labor, different and controversial results were obtained. Serum P53 level was the best for prediction of preterm delivery, as it is the most sensitive for prediction of PTL (sensitivity = 78.6%), while, power Doppler indices of the uterine artery (RI and PI) are the least sensitive (RI sensitivity = 23.8% and PI sensitivity = 19.1%). Also, cervical length had sensitivity of 33.3% and EMG positive parameters had sensitivity of 40.5% but the last are the most specific for prediction of PTL (specificity=100%) within one week from the EMG study. Finally, we recommend additional studies focusing on the problem of preterm birth prediction.

CONFLICT OF INTEREST

There are no conflicts of interest.

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