ROLE OF BIOCHEMICAL MARKERS AND TRANSVAGINAL ULTRASOUND IN THE PREDICTION OF PRETERM LABOR

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ABSTRACT

Background : Evaluation of cervicovaginal fetal fibronectin was considered as one of the most predictive factor for preterm labor when it is 50 ng/ml. Also, Salivary estriol (E3) has the same predictive factor for preterm labor when it is over 2.1ng/ml. The last factor in this study was measurement of cervical length.

Objective: Assessing cervicovaginal fetal fibronectin and maternal Salivary estriol (E3) as biochemical markers, as well as transvaginal ultrasound in the prediction of preterm labor.

Patients and Methods: The study included 150 pregnant women at gestational ages between 24 weeks and 34 weeks confirmed by sure history of last menstrual period and first trimestric ultrasound examination.The patients in this study were fulfilling the inclusion criteria, and were enlisted in a prospective clinical trial. As regard cervicovaginal fetal fibronectin, measure of it as Dacron swab was rotated in posterior fornix of the vagina or ectocervix and sent for assay of fetal fibronectin.

Salivary estriol (E3) was collected from mouth after washing it, and stored till time of assay. Cervical length was evaluated by transvaginal ultrasound.

Results: There was a highly significant difference between women with positive and negative fibronectin regarding gestational age at delivery. Salivary estriol (E3) showed a highly significant difference between women with positive and negative E3 regarding gestational age at delivery. Also, cervical length showed a highly significant difference between women with short and normal cervical length regarding gestational age at delivery. There was no significant effect for age, parity, presence of uterine contractions and uterine anomalies on the outcome of pregnancy .

Conclusion: Cervicovaginal fetal fibronectin, salivary estriol E3 and measurement of cervical length were more helpful in prediction of preterm labor.

INTRODUCTION

The diagnosis of spontaneous preterm labor and accurate prediction of preterm delivery is notoriously difficult. Identification of effective risk assessment markers can potentially improve outcomes by enabling targeted therapy while allowing efficient use of resources and avoiding unnecessary interventions. Considerable progress has been made in the development of accurate methods as fetal fibronectin (FFN) and cervical length assessment to predict preterm birth (PTB) in both symptomatic and asymptomatic high risk women **(Barber et al., 2010).**

As risk assessment tools, both history and CL assessment have their limitations, but when used in conjunction with biochemical markers (such as FFN), prediction is improved. The Preterm Prediction Study demonstrated that if risk factors (previous SPB, CL less than 25 mm and positive FFN) were present, the risk of SPB before 32 weeks was 50 % (**Hutcheon et al., 2012).**

Of the three major placental estrogens, estriol (E3), estradiol (E2), and estrone (El), E3 is the most abundant during late pregnancy. The feasibility of measuring salivary E3 in detecting increased risk of preterm labor has been studied. Salivary E3 level at or above 2.1 ng/mL predicted preterm labor which is more accurately than traditional risk assessment methods (**Sotiriadis et al., 2010).**

The clinical use of fetal fibronectin sampling was significantly improved with the introduction of a rapid, semiquantitative testing protocol, the Tli System (Adeza Biomedical, Sunnyvale, California). Before introduction of the Tli, analysis required specimen transportation to a central laboratory and ELISA testing.

Multiple studies have provided evidence that a positive fetal fibronectin is a redictor of preterm delivery in patients presenting with preterm uterine contractions. A negative fetal fibronectin test identifies patients at low risk for preterm delivery, although a positive test has a limited positive predictive value (**Souka et al., 2011).**

Ultrasonographic evidence of increased internal cervical os diameter has been reported by various researchers as being consistent with cervical incompetence. **Mclaren et al. (2015)** have reported that width of the internal cervical os greater than 1.9 cm was suggestive of cervical incompetence. In another study, has observed that a mean internal os diameter of about 1.6 cm or more was consistent with cervical incompetence (**Lim et al., 2009**). However, these studies did not consider the effect of progressive gestational age on the diameter of the internal os. And associates stated that an internal os width greater than 1.5 cm in the first trimester or greater than 2 cm during the second trimester indicates incompetence (**Peng et al., 2015**)**.**

The present study aimed to assess cervicovaginal fetal fibronectin and maternal salivary estriol (as biochemical markers) as well as transvaginal ultrasound in the prediction of preterm labor.

PATIENTS AND METHODS

This study was a prospective study conducted at Al Azhar University Hospital and Shibin El Khom Teaching Hospital between April 2015 and April 2017.

The study included 150 pregnant women at gestational ages between 24 weeks and 34 weeks confirmed by a sure history of last menstrual period and first trimestric ultrasound examination.

Inclusion criteria: Women in the study were selected with risk factor(s) of preterm birth including:

Current evidence of threatened preterm labor diagnosed by the occurrence of two or more uterine contractions during 10 minutes or less with cervical dilation of 4cm or less, and intact membranes with or without cervical effacement.

Known cervical incompetence by early ultrasound.

● Previous preterm birth.
● Multiple pregnancies.
● Known uterine anomaly such as septate or bicornute uterus.
● Previous cervical surgery (trachelor-raphy or amputation).

Exclusion criteria: Women of ruptured membranes.

Methodology:

I. Cervicovginal fetal fibronectin: Each subject was first examined with sterile vaginal speculum. A Dacron swab was rotated in the posterior fornix of the vagina or ectocervix, and sent for assay of fetal fibronectin. The presence or absence of fetal fibronectin was measured by qualitative test and results were expressed as positive or negative.

II. Salivary estriol (E3) : Women were instructed to wash their mouth with water and to wait for 10 minutes to allow saliva without stimulus, then saliva was collected into standard plastic jars during day time hours (9 am – 8 pm) to avoid diurnal variation.

The patient was instructed to abstain from eating, drinking, smoking or chewing gum for at least one hour before specimen collection and specimens frozen within 4 hours after collection and stored at – 20?C till time of assay.

III. TVU Cervical length Measurement: It was performed using 7.5 MHZ endovaginal transducer (voluson p8).

A standardized measurement of cervical length was followed:

1. The patient emptied her bladder, she then lie in the dorsal lithotomy position.

2. Endovaginal probe was covered with a condom, then inserted.

3. The probe was placed in the anterior fornix of the vagina.

4. A sagittal view of the cervix was obtained showing the long axis view of echogenic endocervical mucosa along the length of the canal.

5. The probe was withdrawn until the image became blurred, and then was re-advanced just enough to restore the clear image.

6. The image was enlarged until the cervix occupied at least two-thirds of the screen, and the internal and external os were well seen.

7. The cervical length was measured from the internal to the external os along the endocervical canal.

8. At least, 3 measurements were obtained and the shortest was taken.

9. Gentle transfundal pressure was applied for 15 seconds, and the cervical length was measured again at least 3 times.

10. The examination lasted at least 5 minutes. The shortest measurement was only recorded for clinical management.

Statistical analysis: Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis, (version 20; Inc., Chicago. IL USA).

Chi-square test was used to measure association between qualitative variables.

Student's t-test was used to compare mean and SD of 2 sets of quantitative normally distributed data.

Pearson's correlation was used to study correlation between two variables having normally distributed data. Sensitivity, specificity, +ve and –ve predictive values, and diagnostic accuracy were calculated.

P-value ≤ 0.05 was considered significant .

RESULTS

Mean age of the studied group was 28.05 ± 4.4, 78% of the studied group were multipara, 66% of the studied group were pregnant in single baby, 56% of the studied group had uterine contractions and 79.3% of the studied group had no uterine anomalies (Table 1).

Table (1): Descriptive data of the studied group.

variables	Number	%
Age (years) Range Mean± SD	19-36 28.05±4.4	
Parity Primi Multi	33 117	22.0 78.0
No. Of fetuses Single Twins	99 51	66.0 34.0
Uterine Contractions Yes No	84 66	56.0 44.0

Thirty six percent of the studied group had positive fibronectin, and 37.3% of the studied group had positive E3 (Table 2).

Table(2): Cervicovaginal fetal fibronectin and salivary E3 .

Variables	Number	%
Fibronectin Positive Negative	54 96	36.0 64.0
E3 Positive Negative	56 94	37.3 62.7

62.7% of women in the study had short cervix <25 mm, (Table 3).

Table (3): Cervical length.

Cervical length	Number	%
<25mm	94	62.7
>25mm	56	37.3

There was a highly significant difference between women with positive and negative E3 regarding gestational age at delivery (Table 4).

Table (4): Relation between E3 result and gestational age at delivery.

E3 / Delivery (weeks)	Negative (no =94)	Positive (no= 56)	P VALUE
Range Mean± SD	24-39 34.50±4.1	25-35 30.8±2.62	< 0.001

There was a significant relationship between outcome of pregnancy and number of fetuses as 19.6% of women with twins pregnancy completed their pregnancy, while 80.4% of women with twins pregnancy delivered preterm. This difference was statistically highly significant. On the other hand, there was no significant difference in the outcome between women with singleton pregnancy. Additionally, there was no significant effect for age, parity, presence of uterine contractions, or uterine anomalies on the outcome of pregnancy (Table 5).

Table(5): Outcome of pregnancy and patient variables.

Outcome / variables	Term		Preterm		P value
Age Range Mean +SD	20-38 28.4+4.7		19-36 27.7+4.2		>0.05
Parity Primi (n=33) Multi (n=117)	14 43	42.4 % 36.8%	19 74	57.6% 63.2%	> 0.05
No. of fetuses Single(n=99) Twins (n=51)	47 10	47.5% 19.6%	52 41	52.5% 80.4%	<0.001
Uterine Contractions Yes (n=84) No (n=66)	31 26	36.9% 39.4%	53 40	63.1% 60.6%	> 0.05
Uterine Anomalies Yes (n=31) No (n=119)	12 45	38.7% 37.8%	19 74	61.3% 62.2%	> 0.05

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There was a significant relationship between +ve salivery E3 and preterm birth as 96.4% of women with +ve salivery E3 delivered preterm in contrast to only 3.6% who delivered at term. When salivery E3 was –ve there was no statistical difference in the outcome (Table 6).

Table (6): E3 of the studied groups and outcome of pregnancy.

Outcome / E3	Term		Preterm		P value
	No	**%**	**No**	**%**	
Positive (56)	(2)	3.6%	(54)	96.4%	
Negative (94)	(55)	58.5%	(39)	41.5%	<0.01

No. of cases were in between brackets.

There was a significant relationship between fetal fibronectin and delivery within 2 weeks and more (Table 7).

Table (7): Fibronectin of the studied group and delivery within 2 weeks and more.

Birth within 3 weeks / Fibronectin	1 week		1 -2 weeks		>2 weeks		P value
50-54 (16)	2	12.5%	5	31.2%	9	56.3%	<0.05
55-60 (18)	6	33.3%	9	50%	3	16.7%	
➢ >60(20)	10	50%	6	30%	4	20%	

Fibronectin was with sensitivity of 47%, specificity of 82%, positive predictive value(PPV) of 81%, negative predictive value(NPV) of 49%, and its accuracy of 61%, salivary estriol(E3) with sensitivity of 58%, specificity of 100, PPV of 100, NPV of 72, and its accuracy of 80, cervical length with sensitivity of 68%, specificity of 86, PPV of 89%, NPV of 62, and it's accuracy of 75% (Table 8).

Table (8): Sensitivity and specificity of fibronectin, E3 and cervical length in predicting PTD.

Parameters / Variables	Sensitivity	Specificity	PPV	NPV	Accuracy
Fibronectin	47%	82%	81%	49%	61%
E3	58%	100%	100%	72%	80%
Cervical length	68%	86%	89%	62%	75%

There was non-significant negative correlation between cervical length and fibronicten also there is a significant positive correlation between fibronectin and E3 (Table 9).

Table (9): Correlation between salivary E3, fetal fibronectin and cervical length.

Correlation / Fibronectin	R	P value
E3	0.263	0.001
Cervical length	0.067	0.425

There was a significant positive correlation between cervical length and E3 (Table 10).

Table (10): Correlation between salivary E3 and Cervical length.

	E3	
	r	**P value**
Cervical length	**0.225**	**0.001**

There was a significant relationship between Uterine contractions and outcome of pregnancy single – twins (Table 11).

Table (11): Uterine contractions of the studied group and outcome of pregnancy.

Outcome					
	Term		**Preterm**		**P value**
Uterine contraction Positive (84)	46	55%	38	45%	<0.001
Negative (66)	11	17%	55	83%	

There was a significant relationship between short cervix and preterm birth as 91.5% of women with short cervix (<25mm) delivered preterm in contrast to only 8.5% who delivered at term.Similary, 87.5% of women with normal cervical length (>25mm) delivered at term in contrast to only 12.5% who delivered preterm (Table 12).

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Table (12): Cervical length of the studied group and outcome of pregnancy.

Outcome					
Cervical length	**Term**		**Preterm**		**P value**
< 25 (N=94)	8	8.5%	86	91.5%	<0.001
25 (N=56) >	49	87.5%	7	12.5%	

There was a highly significant difference between women with positive and negative fibronectin regarding gestational age at delivery (Table 13).

Table (13) : Relation between fetal fibronectin and gestational age at delivery.

Fibronectin			
	Negative (no =96)	**Positive (no= 54)**	**P VALUE**
Delivery (weeks) Range Mean± SD	24-39 35.50±5.01	25-38 32.6±4.62	0.002

DISCUSSION

Fibronectin is often found in the cervicovaginal secretions before 16-18 weeks of pregnancy, and again at the end of normal pregnancy as labor approaches. It is not normally present in cervicovaginal secretions between 22 and 37 weeks. Fibronectin found in cervicovaginal secretions after 22 weeks is a marker of disruption of the decidual-chorionic interface. It has been associated with a six-fold increased risk of preterm birth before 35 weeks and a 14-fold increased risk of preterm birth before 28 weeks **(Malamud, 2011) .**

In the present study, the mean gestational age of delivery for cases with positive fibronectin was 32.6|± 4.62| weeks, while the mean gestational age of delivery for cases with negative fibronectin was 35.5|±5.01| weeks with a statistically significant difference .This mean gestational age for cases with positive fibronectin give chance for adjuvants measurements.

In the present study, it was found that positive cervicovaginal fibronectin (level ≥ 50 ng /ml) was predictive of preterm birth with sensitivity of 47% , specificity of 82%, positive predictive value of 81%, and negative predictive value of 49%. This was in contrast with the study of **Owen and Melissa (2012)** who showed that positive cervicovaginal fibronectin (level ≥ 50 ng /ml) was predictive of preterm birth with a sensitivity of 73% , specificity of 72% , positive predictive value of 25 %, and negative predictive value of 95%.

In the present study, increase in salivary E3 was observed 2-3 weeks before delivery and this was in agreement with **Mclaren et al. (2015)** who showed that E3 had a surge approximately 3 weeks before delivery.

Although digital examination of the cervix is largely imprecise, ultrasound evaluation of cervical length and membrane status can provide useful and reproducible informations. Abdominal ultrasound attempts at cervical evaluation are significantly affected by both habitus and bladder filling which, however, do not affect endovaginal imaging. Thus, an appropriate endovaginal image can be obtained in more than 95% of patients. In the present study, cervical lengths of greater than 25 mm at 25 weeks' gestation had a negative predictive value of 87.5% for preterm birth. Further, as cervical length decreases, the positive predictive value and sensitivity for preterm delivery increase**.** This was in agreement with study of **Lim et al. (2009**) who showed that CL greater than 30 ml at 24 weeks' gestation had a negative predictive value of 97 %**.**

In the current study, transvaginal ultrasound was more sensitive ($\geq$ 60%) in women with increased risk of preterm birth which included women with history of preterm birth or mainly uterine contractions or previous cervical conization. This was in agreement with the study of **Craigo** (**2011**) who showed that transvaginal ultrasound is more sensitive($\geq$ 50%) in women with previous cervical conization**.**

In this study, positive fetal fibronectin (50 ng/ml or more) in patients with symptoms suggestive of preterm labor has been associated with increase in birth within 7-14 days of test, but there was no agreement with study of **Heinne et al. (2007)** Who showed that the positive predictive value for delivery within week was 18% .

In the present study, minimal increases in fFN(l - 54 ng/mL) were associated with a modest increase in the relative risk of sPTD at late preterm GA (34 and 35 weeks). Moderate increases in fFN (55-60 ng/mL) were associated with significant increase in sPTD risk at earlier and wider GA (from through 37 weeks), and the increase levels of fFN (60 ng/mL) were associated with the highest in prediction of PTD. There was no agreement with **Peng et al. (2015**) who showed that minimal increase was at 1- 49 ng/mL, moderate at 50-199 ng/mL, and highest more than 200 ng/mL.

In the present study, the mean gestational age at delivery for cases with positive E3 was 30.8 $\pm$2.62 weeks, and the mean gestational age at delivery for cases with negative E3 was 34.50 $\pm$4.1 weeks with a statistically significant difference.

In current study, it was observed that there was a significant positive correlation between E3 and fibronectin. The increase in cervicovaginal fetal fibronectin was associated with increase in salivery E3 in a strong correlation. On the other hand, there was non significant negative

correlation between cervical length and fibronectin.

CONCLUSION

Cervicovaginal fetal fibronectin, salivary estriol E3 and measurement of cervical length were more helpful in prediction of preterm labor.

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دور الدلالات الكيميائية الحيوية والأشعة التليفزيونية المهبلية في التنبوء بحدوث الولادة المبكرة

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خلفية البحث: إن تقييم نسبة الفيبرونيكتن الجنيني في السوائل المهبيلية يعتبر واحدا من أهم العوامل للتنبؤ بالولادة المبكرة عندما تكون نسبته ٥٠ نانو جرام\ مل أو أكثر وكذلك تكون نسبة الإستريول اللعابي عندما تكون نسبته 2.1 نانوجرام\ مل ويضاف إليهم قياس عنق الرحم.

الهدف من البحث: تقييم نسبة الفيبرونيكتن الجنيني في السوائل المهبلية ونسبة الإستريول اللعابي كعلامات كيميائية مع قياس طول عنق الرحم بالأشعة التليفزيونية المهبلية للتنبؤ بالولادة المبكرة.

المرضى وطريقة البحث: تحتوي الدراسة على ١٥٠ سيدة من الحوامل ما بين الأسبوع ٢٤ إلي ٣٤ الذي تم تحديده مسبقا بالأشعة التليفزيونية في الثلاثة شهور الأولي أو التأكد من أول يوم في أخر للطمث.

ويكون المرضي في هذه الدراسة بهن خواص محددة حتى يتم ضمهن لهذه الدراسة، فمثلا: تم قياس نسبة الفيبرونيكتن الجنيني في السوائل المهبيلية عن طرق أخذ العينة بواسطة مجس مهبلي ووضعه في الجزء الخلفي لعنق الرحم أو داخل عنق الرحم وأرسلت العينة للتحليل، أما بالنسبة لقياس نسبة الإستريول اللعابي لقد تم تجميع العينة من فم السيدات الحوامل بعد تنظيف فمهن بالماء والإنتظار لمدة عشرة دقائق وتخزين العينة حتى يتم تحليلها وكذالك قياس عنق الرحم بواسطة الأشعة التليفزيونية المهبلية.

النتائج:

لقد وجد إختلاف في نسبة الولادة المبكرة في السيدات اللواتي كانت عيناتهن من الفيبرونيكتن والإستريول اللعابي إيجابية عن السيدات التي كانت عيناتهن سلبية.

ولقد وجد أيضا أن هناك اختلاف في نسبة الدلاله بين قصر عنق الرحم وطوله في التنبؤ بحدوث الولادة المبكرة وانه كلما قصر عنق الرحم كلما زادت نسبة حدوث الولادة المبكرة وهذا بغض النظر عن عمر السيدة الحامل وعدد مرات الولادة ووجود إنقباضات رحمية من عدمها.

الإستنتاج:

قياس الفيبرونيكتن الجنيني في السوائل المهبلية والإستريول اللعابي وكذالك قياس طول عنق الرحم يعد من أقوي المؤشرات للولادة المبكرة.