Cervico-vaginal fluid prolactin in premature rupture of membranes:

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Abstract:

Background: Pre labour rupture of membranes (PROM) is one of the most common complications of pregnancy that has a major impact on pregnancy outcome. The assessment of women with possible membrane rupture is management issue faced in every day practice.

Objective: To evaluate the diagnostic power of the vaginal fluid prolactin concentration in pre labour rupture of membranes.

Patients and Methods: A total of 100 pregnant women were recruited in this study. The PROM group consisted of 50 pregnant women between 24 and 41 weeks gestation with confirmed PROM by amniotic fluid pooling and Nitrazine paper test. A control group consisted of 50 pregnant women with similar gestational age without any complaint or complication. All patients underwent speculum examination for amniotic fluid pooling, Nitrazine paper test, vaginal fluid sampling was done then presence of prolactin in this fluid was measured by ELISA.

Follow up of pregnancy outcome to detect gestational age at birth and time interval between sampling and delivery then statistical analysis of data has been performed to determine the reliability of vaginal fluid prolactin in diagnosis of pre labour rupture of membrane.

Results: Vaginal fluid prolactin were significantly different between the two groups ($P < 0.001$) being higher in the PROM group than the control group.

Conclusion: Prolactin in cervico-vaginal fluid may be helpful in the diagnosis of pre labour rupture of membranes

Key wards: prolactin , Cervico-vaginal fluid , pre labour rupture of membranes,

Introduction:

Pre labour rupture of membrane (PROM) is the rupture of the fetal membrane with leakage of amniotic fluid before the onset of labour (i.e in the absence of uterine activity) (1). PROM occur in 8-20% of term pregnancies (2, 3) and 2-25% of all pregnancies (4) .PROM has been shown to be the cause of 18-20% of prenatal mortalities (3) and 21.4% of prenatal morbidity (5, 6, 7) . Pre labour rupture of membrane could be classified as , term pre labour rupture of membrane, Prolonged pre labour rupture of membrane, Pre term pre labour rupture of membrane, and Mid trimester preterm pre labour rupture of membrane (pre-viable PROM) (4) . **Etiology and risk factors of pre labour rupture of Membranes:**

1.Infection of the vagina, particularly during the second trimester. 2. Uterine over distention. 3. Previous PROM. .4. Cervical Incompetence. 5. Smoking. 6. Hormone (8,9,1011,12,13) . **Consequences of PROM:**

1. Infection. 2.Preterm birth. 3.Placenta abruption (14). **Prolactin:** Prolactin (PRL) is produced by anterior pituitary, decidua, myometrium, breast, lymohocytes, leukocytes and prostate. It is a 199-aminoacid single polypeptide chain and it is encoded by a single gene located on the short arm of chromosome 6 (15, 16), It is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. The biological half-life of prolactin in humans is around 15-20 minutes.

Amniotic fluid prolactin:

Amniotic fluid concentrations of prolactin parallel maternal serum concentrations until the 10th week of pregnancy, rise markedly until the 25th week, and then decrease (3000- 5000ng\ml at 19-25 weeks of gestation to levels around 500 ng\ml between 35-41 th weeks), the prolactin level in the amniotic fluid are 5 to 10 times higher than those in the maternal plasma as maternal prolactin does not pass to the fetus in significant amounts, indeed, the source of amniotic fluid prolactin is neither the maternal pituitary nor the fetal pituitary (17). Studies of in vitro culture systems indicate a primary decidual source of prolactin with transfer via amnion receptors to the amniotic ,this decidual synthesis of prolactin is initiated and controlled by progesterone and estrogen not by dopamine, but once decidualization is established. Prolactin secretion continues in the absence of both progesterone and estradiol (18). Amniotic fluid prolactin plays a role in modulating electrolyte and protects the human fetus from dehydration by control of salt and water transport across the amnion. No clinical significance can be attached to maternal and

fetal blood levels in abnormal pregnancies. (19, 20).

Patients and Methods:

A case control study was conducted at Al-Elwiya Maternity Teaching hospital in Baghdad from 10th of February 2016 to the 10th of December 2016. The study was carried on 100 pregnant ladies. The study sample was divided in two groups; the PROM group consisted of 50 pregnant women between 24 and 41 weeks of gestation with diagnosis of PROM confirmed which was by amniotic fluid pooling and nitrazine paper test. The control group consisted of 50 pregnant women with similar gestational age without any complaint or complication. The works up included: 1. History taking about (gestational age, amount of watery vaginal discharge, vaginal bleeding if any, fever and increasing lower abdominal pain). 2. Maternal vital signs (pulse rate, blood pressure and temperature). 3.Cardiovascular and respiratory examination. 4.Obstetrical examination (abdominal examination) for fundal height detection and presence of uterine contraction. In emergency room and under a septic condition, each patient submitted to the test in lithotomy position, A sterile speculum (cuscous) examination was performed after the mother has rested supine for 20-30 minutes. Amniotic fluid can be seen pooling in the posterior fornix, either spontaneously or after fundal pressure, leakage of fluid was inspected and results were registered as positive or negative. And to assess cervical dilatation and length. Nitrazine test (MACHEREY-NAGEL GmbH & Co, Germany):

A cotton tip applicator was inserted in deep vagina and was immediately transferred on nitrazine paper if it turns from yellow to blue, means the membrane was ruptured. 5. Routine investigation (hemoglobin concentration, blood group and RH, white blood cell count, serum glucose, virology screen, GUE) and other investigations as vaginal swab for direct staining for infection.6.Ultrasound examination (Philips HD11.XE wieh frequencies 2-5 MHZ) for gestational age determination and amniotic fluid index (AFI) calculations (21). Gestational age was established according to last menstrual period, or with early pregnancy ultrasound when available. The sample was collected in consultancy clinic at Elwiya Maternity Teaching hospital by direct interview on Sunday and Monday in every week and fulfilling of prepared questionnaire, which was designed for the study.

In control group, 3ml. of normal saline was injected in to the posterior fornix of vagina and then aspirated after1-3 minutes, while the patients in PROM group; direct aspiration of leaking liquor fluid was done during speculum examination.

The aspirated fluid collected in sterile plastic test tubes (10 ml test tube, 18mm*100mm, round bottom, with snap cap) and send immediately to the laboratory. Prolactin levels in the aspirated fluids from all women in both groups were measured by ELISA method (direct ELISA kit-prolactin (ACCU bind Lake forest, CA92630, USA), in room temperature, 100 microliter of Prolactin Enzyme Reagent was added to 25 microliter of the aspirated vaginal fluid sample and waited 15 minutes, then added 300 microliter of washing buffer to the sample 3times and waited 15 minutes then measured prolactin level, the results were obtained after 2 hours and recorded as positive if > 5 ng/ml and negative if < 5 ng/ml. The follow up of pregnant ladies started from 1st visit until delivery. The schedule of visits was according the condition of the patient; The PROM group was assessed as in-patient admission daily by vital signs, uterine contractions, changes in vaginal discharge and fetal well-being to the patients received antibiotics and steroid if less than 37 weeks. The control group was assessed every 2 as out patient weeks about the date of delivery

Statistical analysis:

SPSS version 20 was used for data entry and analysis, Descriptive statistics presented as (mean and standard deviation), frequencies and percentages were used to describe the continues data, student T test for independent samples and chi-square test were used for data analysis, $p \leq 0.05$ was considered significant.

Results:

Table 1 shows distribution of pregnant women according to age that show most of them (48%) arranged between 20-30 years. According to parity, 58% of them between 4-6. According to gestational age, 31% of them ≥ 37 weeks. According to status of membrane, 50% intact and 50% rupture. According to gestational age at birth most of them (61%) more than 37 weeks. According to prolactin level, 50% less than 5 ng\ml (negative) and 50% more than 5ng\ml (positive).

Table (1): Classifications of pregnant women according to age, parity, gestational age at examination, status of membrane, gestational age at birth and prolactin level.

Table 2 shows the relation between prolactin level and age of pregnant women .There was no significant relation between prolactin level and age of pregnant women among different age groups *(P* value 0.195).

Table3 shows the relation between prolactin significant relation between prolactin level and pregnant parity among different parity group (*P* value 0.625).
Table 4

shows the relation between prolactin level and gestational age at examination. There was no significant relation between prolactin level and gestational age at examination among different gestational age groups (*P* value 0.535).
Table5: shows the relation between prolactin level and status of membrane .There was a significant difference in prolactin level in both groups (*P* value <0.001) it was higher in the PROM group than the control group.
level and pregnant parity. There was no Shows the relation between prolactin level and gestational age at birth .There was a significant relation between them (p-value<0.001), which mean that a high prolactin level is much more associated with premature delivery compared to low prolactin level.
This table shows the relation between status of membrane and gestational age at birth .There was a significant difference between them (*P* value<0.001) this mean incidence of prematurity was higher in PROM group than the control group.

Variables	**Frequency**	**%**	
Pregnant age (years)	<20	12	12
	20 – 30	48	48
	>30	40	40
Pregnant parity	0	14	14
	1 - 3	21	21
	4 - 6	58	58
	>6	7	7
Gestational age at examination (weeks)	24 – 27	23	23
	28 – 30	23	23
	31-36	23	23
	≥37	31	31
Status of membrane	Intact	50	50
	Ruptured	50	50
Gestational age at birth (weeks)	<37	39	39
	≥37	61	61
prolactin level	negative	50	50
	Positive	50	50

Table 2 Association of prolactin level with age

Prolactin level	**Pregnant age**			**Total**	**P-value**
	<20 years	**20-30 years**	**>30 years**		
negative	4	22	24	50	0.195
positive	8	26	16	50	
Total	12	48	40	100	

Table 3 Association of prolactin level with parity

Prolactin level	**Pregnant parity**				**Total**	**P-value**
	0	**1-3**	**4-6**	**>6**		
negative	8	10	28	4	50	0.625
positive	6	11	30	3	50	
Total	14	21	58	7	100	

Table 4: Association of prolactin level with gestational age at examination

Prolactin level	Gestational age at examination (weeks)				Total	P-value
	24-27	28-30	31-36	≥37		
negative	11	12	12	15	50	0.535
positive	12	11	11	16	50	
Total	23	23	23	31	100	

Table 5 Association of prolactin level with status of membrane

Prolactin level	Status of membrane		Total	P-value
	Ruptured	Intact		
negative	0	50	50	<0.001
positive	50	0	50	
Total	50	50	100	

Table 6: Association of prolactin level with gestational age at birth

Prolactin level	Gestational age at birth (weeks)		Total	Chi Square Test	P-value
	<37	≥37			
negative	5	45	50	42.0	<0.001
positive	34	16	50		
Total	39	61	100		

Table 7:Association of status of membrane and gestational age at birth.

Status of membrane	Gestational age at birth (weeks)		Total	Chi Square Test	P-value
	<37	37+			
Ruptured	34	16	50	42.0	<0.001
Intact	5	45	50		
Total	39	61	100		

Discussion :

A timely and accurate diagnosis of PROM is critical to optimize perinatal outcome and minimize serious complications such as cord prolapse and infections including chorioamnionitis and neonatal sepsis ([22, 23]. In most cases diagnosis is made according to the clinical complaints and traditional methods [24]. However, clinical complaint of patient is not reliable [25].With the possible exception of direct visualization of amniotic fluid spurting from the cervical os, all clinical signs have limitations in terms of diagnostic accuracy, cost and technical case. Moreover, reliance on clinical assessment alone leads to both false-positive and false–negative results [2] . Thus, we need simple, reliable and rapid tests for diagnosis of PROM. Since there is no unique and noninvasive gold standard test applicable to all patients with 100% accuracy several biochemical markers have been studied previously [25]. Despite the improved diagnostic value of these markers, they have not become popular because of their complexity and cost [24]. This study showed that diagnostic power of prolactin for PROM was in acceptable range. As far as we know, limited studies related to PROM and vaginal fluid prolactin concentrations have been published so far.In our study, found that prolactin concentrations in vaginal fluid were significantly higher in the PROM group than in the control group. ($p<0.001$). This is consistent with results of study was done by Shahin and Raslan (2007) [26] . The purpose of that study was to determine the effectiveness of vaginal fluid BhCG, AFP and prolactin measurements in detection of PROM. The results showed that vaginal fluid concentrations of three markers were significantly higher in the PROM group than in the control group [27] also it consistent with results of study done by Karriman *et al.*(2012) found that prolactin level in confirmed PROM showed a highly statistical significant difference when compared to control group ($p<0.001$) [27] . Shahin *et al.* (2007)[26] and Buyukbarak *et al* [28] (2004) utilized the ECLICA method (Electrochemoluminescence assay) to measure prolactin that is more sensitive than ELISA. However, it is an expensive and complex test. Furthermore, it is not available in most laboratories.In this study, we utilized the ELISA method to measure prolactin, because it is available in our laboratories. An ideal laboratory diagnostic technique should be affordable and available. Our study reported low vaginal fluid prolactin in pregnant women with intact amniotic membranes (control group) (range: 0.2-3.6ng/ml) and in PROM, vaginal fluid prolactin levels were significantly high and ranged from 100-200 ng/ml. In contrast Huber *et al.* (1983) assayed the amount of prolactin, AFP and hPL in vaginal washing fluid. Despite the higher concentration levels of the three markers in PROM group, they speculated that, measurement of these proteins in vaginal fluid could not be a suitable clinical test for the diagnosis of PROM. The reason was the presence of considerable overlap between groups and a high rate of false-positives [29].

Kariman *et al.,* (2012). found that No significant statistical difference was observed between the two groups (PROM and Control) as regards age, gestational age and Parity [27] .

Our results are consistent with this study, there was no difference in prolactin level as regards age, gestational age and parity (P-value 0.195, P-value 0.535, P-value 0.625) respectively. In contrast Buyukbayrak *et al.* (2004) assayed that the vaginal fluid prolactin concentration decreased to words term [28]. In our results a high incidence of prematurity in confirmed PROM group which may be due to termination of the pregnancy because of maternal indication (chorioamnionitis) or fetal indication (fetal distress). On the other hand, prematurity in control group was mainly due to spontaneous preterm labor.

Conclusion: Prolactin in cervico-vaginal fluid can indicate pre labor rupture of membranes**.**

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