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Evaluation of Cervico-Vaginal Fluid Prolactin in Premature Ruptured Membranes

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Abstract: ***Premature 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 a management issue that is faced in every day practice. This prospective case control study was conducted at Karama Teaching Hospital in Baghdad from 1-1-2016 to 31-12-2016 on 80 pregnant women (40 PROM group with 24-41 week gestation) and (40 pregnant women with similar gestation age without any compliant or complication as a control group) to evaluate the diagnostic power of the vaginal fluid prolactin concentration in premature rupture of membranes. PROM was confirmed by amniotic fluid pooling and Nitrazine paper test. 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. Levels of vaginal fluid prolactin were shown to be significantly higher (P < 0.001) in the PROM group when compared with the control group. It can be concluded that Prolactin in cervico-vaginal fluid can indicate pre labor rupture of membranes.***

Keywords: PROM, Cervico-Vaginal fluid Prolactin

1. Introduction

Premature rupture of membrane (PROM) is the rupture of the fetal membrane with leakage of amniotic fluid before the onset of labor (i.e in the absence of uterine activity) [1] and can occur at any gestational age [2, 3].

PROM occurs in (8-20%) of term pregnancies [2, 4] and (2-25%) of all pregnancies [5].

PROM has been shown to be the cause of (18-20%) of prenatal mortalities (4) and (21.4%) of prenatal morbidity [6, 7, 8].

PROM is classified into term premature, prolonged mature, pre term mature and mid trimester premature rupture of membrane [5].

The membranes contain many cell types, but are avascular and without nerve cells. The cells appear columnar where the membranes are attached to the placenta, but become more flattened or cuboidal adjacent to the decidua [9].

Amniotic fluid is a clear, and pale straw-coloured fluid which is found within the first 12 days following conception within the amniotic sac and surrounds the growing baby in the uterus [10].

Aminiotic fluid is responsible for protecting the fetus, temperature control, infection control, lung and digestive system development, lubrication and umbilical cord support [10].

Prolactin (PRL), also known as luteotropic hormone or luteotropin, and in humans, prolactin is produced in the 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 [11, 12].

Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. It is secreted in pulses in between these events. The biological half-life of prolactin in humans is around 15-20 minutes. It stimulates the mammary glands to produce milk (lactation). Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands and prepare for milk production, which normally starts when the levels of progesterone fall by the end of pregnancy and a suckling stimulus is present.

2. Patients and Methods

Our prospective case control study was conducted at Al-Karama Teaching Hospital in Baghdad from 1-1-2016 to 31-12- 2016.

The study included pregnant ladies visiting consultancy clinic at Karama Teaching Hospital in their second and third trimester, for antenatal care or for treating pregnancy symptoms and complications (such as pain, watery vaginal discharge).
The works up included history taking about (gestational age, amount of watery vaginal discharge, vaginal bleeding if any, fever and increasing lower abdominal pain), maternal vital signs (pulse rate, blood pressure and temperature), cardiovascular and respiratory examination, obstetrical examination (abdominal examination) for fundal height detection and presence of uterine contraction, 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, ultrasound examination (Philips HD11.XE wieh frequencies 2-5 MHZ) for gestational age determination and amniotic fluid index (AFI) calculations.

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 and fulfill-

ing of prepared questionnaire form, which was designed to include (Name of patient, age, parity, gestational age at examination, speculum examination, nitrazine test, result of ultrasound, prolactin level, time interval between sampling and delivery, gestational age at birth).

The sample size was 100 pregnant ladies. Only 80 patients were included in our study and 20 were excluded according to exclusion criteria. The study sample was divided in two groups; the PROM group consisted of 40 pregnant women between 24 and 41 weeks of gestation with diagnosis of confirmed PROM by amniotic fluid pooling and nitrazine paper test. The control group consisted of 40 pregnant women with similar gestational age without any complaint or complication. They fulfilled the inclusion criteria.

The inclusion criteria were as follows: Singleton pregnancies between 24-41 weeks, viable fetus. PROM was diagnosed by amniotic fluid pooling on speculum examination and positive nitrazine paper test. The exclusion criteria were as follow: Visible blood in vaginal secretion, use of vaginal suppositories, intercourse in prior night, intra uterine death, presence of fetal anomalies, multiple pregnancies, meconium stained liquor, chorioamnionitis, suspicious PROM, history of medical diseases and regular uterine contractions.

In emergency room and under a septic condition, each patient submitted to the test in lithotomy position, a sterile speculum (cuscous) examination should be 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.

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.

Results obtained were measured at the Karama Teaching Hospital by using Direct ELISA kit- prolactin (ACCU bind Lake forest, CA92630, USA).

In the laboratory, and 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 300 microliter of washing buffer was added to the sample 3 times and waited 15 minutes then prolactin level was measured. The results were obtained after 2 hours and recorded as positive if >5 ng|ml and negative if <5 ng|ml

Statistical Analysis

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

3. Results

Table 1: Association of prolactin level with pregnant women age

Prolactin level	Pregnant age			Total	Chi Square Test	P-value
	<20 years	20-30 years	>30 years			
negative	2	16	22	40	5.89	0.053
positive	6	22	12	40		
Total	8	38	34	80		

The table above shows the relation between prolactin level and age of pregnant women with chi-square test (5.89). There was no significant relation between prolactin level and age of pregnant women among different age groups (P-value 0.053).

Table 2: Association of prolactin level with pregnant parity

Prolactin level	Pregnant age				Total	Chi Square Test	P- value
	0	1-3	4-6	>6			
negative	6	8	24	2	40	0.53	0.768
positive	4	9	26	1	40		
Total	10	17	50	3	80		

Table (2) shows the relation between prolactin level and pregnant parity with chi-square test (0.53). There was no significant relation between prolactin level and pregnant parity among different parity groups (P-value 0.768).

Table 3: Association of prolactin level with gestational age at examination

Prolactin level	Gestational age at examination (weeks)				Total	Chi Square Test	P-value
	24-27	28-30	31-36	≥37			
negative	8	10	10	12	40	0.33	0.945
positive	10	9	9	12	40		
Total	18	19	19	24	80		

This table shows the relation between prolactin level and gestational age at examination with chi-square (0.33) was shown in table (3). There was no significant relation between prolactin level and gestational age at examination among different gestational age groups (P-value 0.945).

Table 4: Association of prolactin level with status of membrane

Prolactin level	Status of membrane		Total	Chi Square Test	P-value
	Ruptured	Intact			
negative	0	40	40	76.00	<0.001
positive	40	0	40		
Total	40	40	80		

The table above shows the relation between prolactin level and status of membrane with chi-square (76.0). There was a highly significant increase in prolactin level (P-value <0.001) in the PROM group when compared with the control group.

Table 5: 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	3	37	40	37.0	<0.001
positive	30	10	40		
Total	3	47	80		

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 as shown in table (5).

4. Discussion

Simple, reliable and rapid tests for diagnosis of PROM are needed. Since there is no unique and noninvasive gold standard test applicable to all patients with 100% accuracy, several biochemical markers have been studied previously [13]. Despite the improved diagnostic value of these markers, they have not become popular because of their complexity and cost [14].

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.

In our study, it was found that prolactin concentrations in vaginal fluid were significantly higher in the PROM group than in the control group.

This is consistent with results of study done by Shahin and Raslan [15] (2007). 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 [15] also it was consistent with results of study done by Kariman et al. who found that prolactin level in confirmed PROM showed a highly statistical significant increase when compared to control group (p<0.001) [16].

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. 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 [17].

Kariman et al. found that No significant statistical difference was observed between the two groups (PROM and Control) in regard to age, gestational age and Parity [16]. Our results are consistent with their study, there was no difference in prolactin level in regard to age, gestational age and parity (P-value 0.195, P-value 0.535, P-value 0.625) respectively. In contrast, Buyukbayrak et al. reported that the vaginal fluid prolactin concentration decreased towards term [18].

Our results showed 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 the control group was mainly due to spontaneous preterm labor.

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