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RESEARCH ARTICLE

Role of vaginal wash prolactin, lactate, and ceruloplasmin in diagnosis of premature rupture of membrane

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ABSTRACT

Objectives: To assessing the effectiveness of prolactin, lactate, and ceruloplasmin in vaginal-washing fluid as diagnostic non-invasive markers of premature rupture of membrane. **Methods**: Eighty pregnant women presented with PROM were randomly selected; speculum test, collection of samples and nitrazine paper test were done .Vaginal washing fluid samples were examined for prolactin, ceruloplasmin and lactate levels by enzyme-linked immunosorbent assay (ELISA). **Results**: Measurement of vaginal wash Lactate levels is more sensitive than both Prolactin and Ceruloplasmin. While the specificity of both Prolactin and Ceruloplasmin in vaginal wash are the same and higher than that of Lactate. **Conclusion:** Measurement of vaginal wash Prolactin, Lactate and Ceruloplasmin with ELISA method is a reliable, and non-invasive test for diagnosis of PROM.

Keywords: Amniotic fluid, Markers, PROM.

The amnion is formed of multiple separate layers; an inner cuboidal epithelial layer bathed in the amniotic fluid, and resting on a basement membrane, this basement membrane is attached to an acellular collagenic compact layer that rests on a layer of fibroblast-like mesenchymal cells. The outermost layer is the zonaspongiosa that slides easily over the chorion [1] which is the thick membranous continuation of the placenta that lines the amnion from outside. As the embryo enlarges in size, both meet at about the 12th week of gestation. The amnion easily strips off the chorion, even at term [2]. Amniotic fluid surrounding the fetus is slightly alkaline (pH 7.0 to 7.5), composed of 98-99% water and 1-2% solids; proteins, glucose, lipids, hormones, enzymes, minerals, and suspended materials [3]. The fetus participates in its production through active secretion from the amniotic epithelium, transudation from fetal circulation, fetal urine and buccal secretion. The mother shares by transudation from the maternal side of the placenta. The exchange of water in the amniotic fluid is very rapid; the exchange from the maternal side is done by transudation through the membranes related to the uterine wall, whereas from the fetus it is mainly by swallowing and urination [4].

Premature rupture of membranes (PROM) refers to

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rupture of the fetal membranes prior to the onset of labor, at any gestational age and is associated with an increased risk of ascending infection [5]. PROM is either a) Prelabor ROM: if occurs before the onset of the labor. b) Preterm PROM: if occurs before thirty-seven completed weeks of gestation [6]. It can be explained by different factors. This is a normal process if occurs at term preparing for labor and delivery. But, it is a problem when it occurs pre-term (before 37 weeks). The natural weakening of fetal membranes is thought to be due to one or a combination of :1) Programed cell death releasing chemical markers detected in higher concentrations in cases of PPROM. [7] 2) Poor assembled collagen: In cases of PPROM, proteins that bind and cross-link collagen to increase its tensile strength are altered, leading to breakdown of collagen by enzymes called matrix metalloproteinases (MMPs), found at higher levels in PPROM amniotic fluid. Matrix metalloproteinases are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs), which are found at lower levels in PPROM amniotic fluid [8]. In premature rupture of membranes, these processes are activated too early. Infection and inflammation likely explains why membranes break earlier than they are supposed to.

In some studies, bacteria have been found in the amniotic fluid in about one-third of cases of PROM. Even if testing of the amniotic fluid is normal, a subclinical infection may still be contributing. In response to infection, the body creates inflammation by making chemicals (ex: cytokines) that weaken the fetal membranes and put them at risk for rupture [9]. The fetal membranes serve as a barrier to ascending infection. Once the membranes rupture, both the mother and fetus are at risk of infection and of other complications. Fetal risk is related primarily to the gestational age. Preterm PROM is associated with a 4-fold increase in perinatal mortality and a 3-fold increase in neonatal morbidity, including respiratory distress syndrome, poly microbial intra-amniotic infection, intraventricular hemorrhage, and neonatal deaths [10]. Despite initial suggestions, the weight of evidence in the literature suggests that preterm PROM is not associated with acceleration in pulmonary maturation [11]. Other neonatal complications include fetal pulmonary hypoplasia prior to 22 weeks; skeletal deformities related to severity and duration of preterm PROM, cord prolapse especially with non-vertex presentations. Severe oligohydramnios results in cord compression and non-reassuring fetal testing (fetal distress) in labor, leading to increased rate of cesarean delivery. Preterm PROM and exposure to intrauterine inflammation/infection have been associated with an increased risk of neurodevelopmental impairment [12]. Maternal complications may occur as well: clinically evident intra-amniotic infection, and postpartum endometritis, which occurs in 2% to 13% of women with preterm PROM [13-15].

Cases often present before the onset of labor with leaking membrane, in some cases it is easy to diagnose ruptured membranes by a vaginal examination [15]. But in some cases it is not easy if the os is closed or when it is open and the bag of membrane is present with the possibility of a small higher rent in the membrane. In such conditions laboratory diagnosis may help-

1) Nitrazine Test**:** Depends on the change of the normal acidic vaginal pH (4.5-5.5) into alkaline pH due to the presence of amniotic fluid which has an alkaline pH of 7-7.5. Nitrazine paper quickly will turn deep blue if the vaginal fluid has an alkaline pH. The membranes probably are intact if the color of the paper remains yellow or changes to olive yellow. Antiseptic solutions, urine, blood and vaginal infections alter the vaginal pH and cause false positive result. This test is simple, rapid, inexpensive and fairly reliable method. The nitrazine test produces 12.7% false negative and 16.2% false positive results [16, 17].

2) Fern Test**:** Ferning results from the drying out of salts contained in the amniotic fluid. Leading to crystallization of sodium chloride derived from the amniotic fluid. The accuracy of the test is affected by blood or meconium, the test may produce false positive results if the sample is obtained from the cervix, because dry cervical mucus forms arborization. The fern test gives 4.8% false negative and 4.4% false

positive results. The diagnosis of PROM is close to 100% reliable if the vaginal fluid gives both positive nitrazine and positive fern test [18, 19].

However, Intermittent or low volume vaginal discharge or presence of urine or semen may interfere with diagnosis of PROM. Nitrazine and fern tests may also lead to false positive or negative results. A variety of ancilliary techniques for confirmation of membrane rupture have been suggested. Some nonspecific laboratory tests using markers reflecting decidual disruption rather than membrane rupture [20]. Several markers have been studied, including insulin like growth factor binding protein 1 (IGFBP 1) [21] , alpha-fetoprotein (AFP) [17], prolactin, beta subunit of human chorionic gonadotropin (β hCG) [22], creatinine, urea [23], lactate [24] and placental alphamicroglobulin 1 (PAMG 1) [25]. Also the sonographic identification of oligohydramnios developing after membrane rupture facilitates diagnosis and management [26, 27].

Some invasive tests were also suggested including: Transabdominal injection of dye (indigo carmine, Evans blue, fluorescein) into the amniotic cavity [28], Amnioscopy for direct visualization of the membranes and the AF [29-31]. An ideal test has not yet been proposed which should be simple, rapid, inexpensive, and non-invasive. Optimally, the accuracy of the test should not be hampered by the presence of blood, semen, infected urine, or other contaminants. An accurate biochemical marker for membrane rupture should have a high concentration in the amniotic fluid, a low concentration in maternal blood, and an extremely low background concentration in cervico-vaginal discharge with intact membranes. Ceruloplasmin is a known plasma antioxidant that increases in concentration during inflammation. There is an association of Ceruloplasmin in cervicovaginal secretions of third-trimester pregnant women and developed PROM and it is likely that the findings reported in this issue are further confirmation of the hypothesis that inflammation plays a role in PROM [32]. This study aims at assessing the effectiveness of prolactin, lactate, and ceruloplasmin in vaginal-washing fluid as diagnostic non-invasive markers.

Methodology

Eighty pregnant women were randomly selected from patients admitted to Northwest El- delta branch Karmouz Elomalli Hospital, in their third trimester with history of vaginal fluid leakage were allocated into the study after signing an informed consent. All patients underwent sterile Cusco speculum examination to detect amniotic fluid leakage and to allow vaginal fluid sample collection, assessement of cervical dilatation and a transabdominal sonography to detect fetal viability gestational age (GA), amniotic fluid index (AFI), placental site and congenital anomalies.

Inclusion Criteria: 1) Gestational age between 28-40 weeks (from LMP or based on a 1st trimester sonography), 2) Singleton pregnancy

Exclusion Criteria: 1) Vaginal bleeding, 2) Uterine contraction, 3) Placenta previa 4) Medical complications that justify termination of pregnancy such as preeclampsia and diabetes mellitus, 5) Fetal congenital anomalies.

Selected women were categorised according to clinical examination and results of nitrazine test into two groups - Group I (Confirmed PROM): Included fourty pregnant women with history of watery fluid leakage, positive fluid leak upon sterile Cusco speculum examination and positive nitrazine paper test with decreased AFI <10. Group II (Control group): Included fourty pregnant women of the same gestational age without any clinical evidence of fluid leakage and with negative nitrazine test. Collection of the sample was done with the patient lying in the lithotomy position speculum testing, the nitrazine test was performed using a swab to obtain a sample from the posterior fornix. The swab was drawn on a strip of nitrazine paper. The color read against the colors and numbers on the nitrazine package. A pH higher than 6.5 considered to represent the rupture of the membranes. Then the posterior fornix was irrigated with 3 cm saline using a sterile syringe. With the same syringe, vaginal washing fluid was aspirated and sent immediately to the laboratory for determination of prolactin, ceruloplasmin, and lactate levels determined

quantitatively by enzyme-linked immunosorbent assay (ELISA) [33-35].

Assessment of vaginal fluid prolactin, ceruloplasmin, and lactate

Sample storage: The vaginal fluid samples were put on ice immediately after aspiration, and then centrifuged for 10 minutes at 3000 rpm as soon as possible. Supernatants were aliquoted and stored at –20°C until further biochemical assay.

Principle of human Prolactin (PRL), Ceruloplasmin (CP) and Lactic Acid (LA) ELISA: The used method assays human PRL, CP and LA levels in the samples, using purified human PRL, purified human CP, and purified human LA antibodies respectively, to coat microtiter plate wells, making solid-phase antibody. After adding PRL, CP or LA to the wells, combined antibody with labeled enzyme, become antibody - antigen - enzyme-antibody complex. After washing, the substrate was added. The substrate becomes blue in color by the action of HRP enzyme. The reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentrations of PRL, CP and LA in the samples were then determined by comparing the O.D. of the samples to the standard curve.

Assay procedure done for prolactin, ceruloplasmin, and lactate: All reagents and samples were brought to room temperature before use.

1) Dilution and addition of Standard: 10 Standard wells were set on the microtiter plate coated, 100µl of standard were added to the first and the second well, then Standard dilution 50µl was added to the first and the second well, after mixing; 100µl were taken from the first and the second well then added to the third and the fourth well separately. Then Standard dilution 50µl was added to the third and the fourth well, after mixing; 50µl were taken from the third and the fourth well separately and discarded. 50µl were taken from the third and the fourth well separately and added to the fifth and the sixth well separately, then Standard dilution 50µl was added to the fifth and the sixth well, after mixing; 50µl were taken from the fifth and the sixth well and added to the seventh and the eighth well, then Standard dilution 50µl was added to the seventh and the eighth well, mixing; 50µl were taken from the seventh and the eighth well and add to the ninth and the tenth well, Standard dilution 50µl was added to the ninth and the tenth well, mixing , 50µl were taken from the ninth and the tenth well and discarded. 50µl were kept in each well after diluting, (density: 1800µg/L, 1200µg/L , 600µg/L, 300µg/L, 150µg/L)

2)Addition of samples: Blank wells were set separately (in blank comparison wells sample and enzyme conjugate were not added, the other steps were same). Sample dilution 40µl was added to sample well, and then 10µl sample were added (sample final dilution is 5-fold). Then gentle mixing was performed.

3)Incubation: After closing the plate with closure plate membrane, incubation for 30 mins at 37^{0}C was done.

4)Preparation of wash solution: 30-fold wash solution diluted 30-fold with distilled water and reserved.

5)Manual Washing: Incubation mixture was removed by aspirating contents of the plate into the sink. Using a squirt bottle, each well was completely filled with wash solution, then contents of the plate were aspirated into the sink. This procedure was repeated four more times for a total of five washes. After final wash, the plate was inverted and blotted dry by hitting it onto absorbent paper until no moisture appeared.

6) Addition of the enzyme: 50µl enzyme conjugate reagents were added to each well, except blank well.

7)Incubation: Same as step 3.

8)Washing: Same as step 5.

9)Color: 50µl substrate A and substrate B were added to each well, covered and incubated for 15 mins at 37^{0}C.

10)Stopping the reaction: 50µl stop solution were added to each well and well mixed.

11)Assay: The optical density of each well was determined within 15mins by a micro plate spectrophotometric reader.

Table 1: Comparison between the two studied groups according to age, gestational age, gravidity, parity, abortions and amniotic fluid index

Categories	Cases (n = 40)	Control (n = 40)	Test of sig.	P
Age (years)				
Min. – Max.	23.0 – 38.0	23.0 – 39.0	t= 0.947	0.347
Mean ± SD.	31.43 ± 3.73	32.38 ± 5.13		
Median	31.50	33.0		
Gestational age (weeks)				
Min. – Max.	30.0 – 39.0	32.0 – 40.0	t= 1.540	0.128
Mean ± SD.	34.65 ± 2.79	35.58 ± 2.58		
Median	34.0	35.50		
Gravidity				
Min. – Max.	1.0 – 3.0	1.0 – 4.0	Z= 1.100	0.271
Mean ± SD.	2.15 ± 0.89	2.40 ± 0.96		
Median	2.0	3.0		
Parity				
Min. – Max.	0.0 – 2.0	0.0 – 3.0	Z= 1.322	0.183
Mean ± SD.	0.98 ± 0.86	1.33 ± 1.12		
Median	1.0	1.0		
Abortions				
Min. – Max.	0.0 – 2.0	0.0 – 3.0	Z= 0.328	0.743
Mean ± SD.	1.08 ± 0.83	1.20 ± 1.07		
Median	1.0	1.0		
AFI (cm)				
Min. – Max.	4.0 – 8.0	12.0 – 18.0	t= 24.307*	<0.001*
Mean ± SD.	6.08 ± 1.35	15.05 ± 1.91		
Median	6.0	15.0		

t: Student t-test; Z: Mann Whitney test; *:Statistically significant at p ≤ 0.05

Calculation of the results for prolactin, ceruloplasmin, and lactate: Four parameters logistic curve fitting program was used for drawing the calibration curve (standard curve) and the sample values were read off the curve. The standard density was taken as the horizontal, the OD value as the vertical. The corresponding density was estimated according to the sample OD value by the standard curve, multiplied by the dilution multiple, or the straight line regression equation of the standard curve was calculated with the standard density and the OD value, with the sample OD value in the equation, the calculated sample density, multiplied by the dilution factor, is the sample actual density.

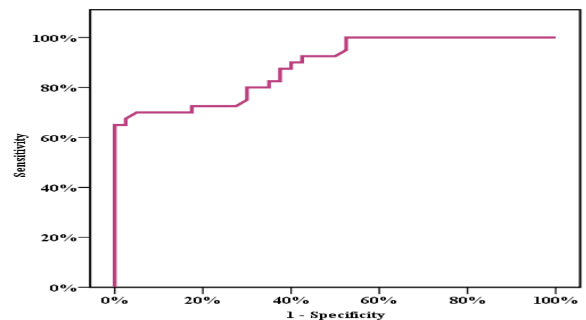
Statistical analysis of the data: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 [36, 37]. Quantitative data were described using range (minimum and maximum), mean, and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, if it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparisons between two independent populations were done using independent t-test. Significance of the obtained results was judged at the 5% level.

Results

No statistically significant difference has been observed between cases and control with respect to age, gestational age, gravidity, parity, and abortions whereas amniotic fluid index showed statistically significant difference (p<0.001) between cases and control (table 1).

There was a statistical significant difference between cases and control regarding the level of vaginal wash Prolactin (P<0.001) (table- 2).

Figure 1: ROC curve for Prolactin to diagnose cases



Receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of Prolactin (ng/l) in PROM cases

Table 2: Vaginal wash Prolactin levels (ng/l), Ceruloplasmin levels (pg./ml) and Lactate levels (µg/L) among the two studied groups				
	Cases (n = 40)	**Control (n = 40)**	**T**	**P**
Prolactin				
Min. – Max.	593.0 – 1023.0	427.0 – 670.0		
Mean ± SD.	721.99 ± 109.25	586.15 ± 63.97	6.786*	<0.001*
Median	700.50	601.50		
Ceruloplasmin				
Min. – Max.	500.0 – 789.50	125.50 –512.0	6.297*	<0.001*
Mean ± SD.	573 ± 76.71	457.99 ± 86.94		
Median	550.0	495.0		
Lactate				
Min. – Max.	2000.5 – 3488.5	1212.0 - 2140.0	9.040*	<0.001*
Mean ± SD.	2462.73±420.09	1751.23±267.06		
Median	2360.0	1761.25		

t: Student t-test; *: Statistically significant at p ≤ 0.05

collectively versus control group. The area under the curve was 0.885 which is significant (P<0.001), this means that vaginal wash Prolactin at cut - off value level of 659 ng/l could significantly predict the occurrence of PROM with diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 70.0%, 95.0%, 93.3%, 76.0% respectively (Figure 1, table 3).

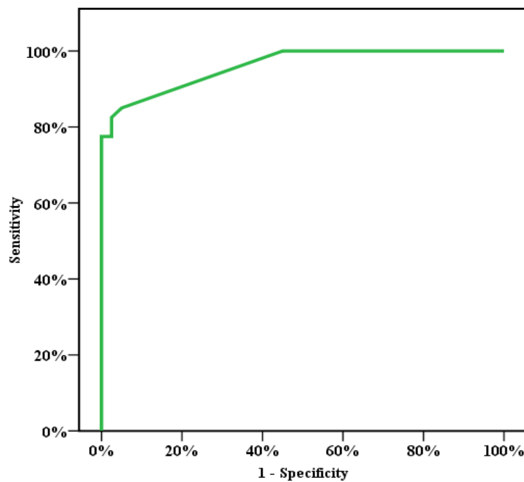


Figure 2: ROC curve for Ceruloplasmin to diagnose cases

Vaginal wash Ceruloplasmin level showed a statistically significant difference (P<0.001) between cases and control. Receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of Ceruloplasmin (pg/ml) in PROM cases collectively versus control group. The area under the curve was 0.960 which is significant (P<0.001), this means that vaginal wash Ceruloplasmin at cut-off value level of 500 pg/ml could significantly predict the occurrence of PROM with diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 85.0%, 95.0%, 94.4%, 86.4% respectively (Figure 2, table 3).

In this study, the vaginal wash lactate range in cases was from 2000.50 – 3488.50 µg/L with a mean value 2462.73 ± 420.09 µg/L, while vaginal wash lactate range in the control was from 1212.0 to 2140.0 µg/L with a mean value of 1751.23 ± 267.06 µg/L. This proves that vaginal wash lactate level showed a statistically significant difference (P<0.001) between cases and control. Receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of Lactate (µg/L) in PROM cases collectively versus control group. The area under the curve was 0.974 which is significant (P<0.001), this means that vaginal wash Lactate at cut-off value level of 2031 µg/L could significantly predict the occurrence of PROM with diagnostic sensitivity, specificity, positive predictive value (PPV),and negative predictive value (NPV) 95.0%, 92.50%, 92.7%, 94.9% respectively.

In PROM group: A significant positive correlation was found between Lactate and Prolactin (r = 0.293,

p<0.067), and Prolactin and Ceruloplasmin (r=0.291,p<0.069).

Table 3: Agreement (sensitivity, specificity and accuracy) for Prolactin, Ceruloplasmin and Lactate

	Youden index	Cutoff	AUC	P	Sensitivity	Specificity	PPV	NPV
Prolactin	0.650	659	0.885*	<0.001*	70.0	95.0	93.3	76.0
Ceruloplasmin	0.800	500	0.960*	<0.001*	85.0	95.0	94.4	86.4
Lactate	0.875	2031	0.974*	<0.001*	95.0	92.5	92.7	94.9

Table 4: Correlation between Prolactin, Ceruloplasmin and Lactate

	Cases		**Control**		**Total sample**	
	R	**p**	**r**	**p**	**R**	**P**
Lactate vs Prolactin	0.293	0.067	-0.012	0.940	0.552*	<0.001
Lactate vs Ceruloplasmin	-0.011	0.947	0.043	0.791	0.422*	<0.001
Prolactin vs Ceruloplasmin	0.291	0.069	0.085	0.604	0.482*	<0.001

r: Pearson coefficient; *: Statistically significant at p ≤ 0.05

A significant negative correlation was found between Lactate and Ceruloplasmin (r=-0.011, p<0.947)

As for control group: A significant negative correlation was found between Lactate and prolactin (r=-0.012, p<0.940), and Lactate and Ceruloplasmin (r=0.043, p<0.791). A significant negative correlation was found between Prolactin and Ceruloplasmin (r=0.085, p<0.604).

Discussion

Premature rupture of membranes (PROM) is a significant obstetrical problem, which can lead to maternal morbidity and imminent term or preterm labour. A timely and accurate diagnosis of PROM is therefore critical to optimize perinatal outcome and minimize complications as cord prolapse and infections including chorioamnionitis and neonatal sepsis [38, 39]. In most cases diagnosis is made according to the patient complaints and traditional clinical methods [40]. However, complaint of patient is not reliable [41]. 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. Moreover, reliance on clinical assessment alone leads to false-positive and false–negative results [42]. 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 [41]. Despite the improved diagnostic value of these markers, they have not become popular because of their complexity and cost [42].

In our study, a cut-off value of 659 ng/l was proposed for PRL and its sensitivity, specificity, positive predictive value, negative predictive value were 70%, 95%, 93.3%, 76% respectively. Our study used human Prolactin ELISA kit (biological fluids) as a method to detect Prolactin in

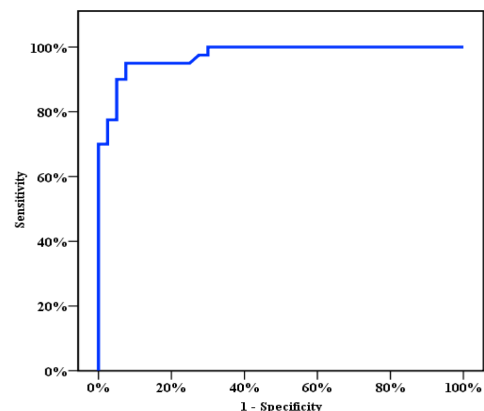


Figure 3: ROC curve for Lactate to diagnose cases

vaginal wash .Other studies also used the same marker. The first study was done by Buyukbayrak et al (2004). In that study 38 patients with confirmed PROM, 32 patients with suspected but unconfirmed PROM and 70 pregnant women without any complaint or complication were included. The sensitivity, specificity, positive predictivity and negative predictivity were 95%, 78%, 93%, 84%, and 87%, respectively in detecting PROM by evaluation of vaginal PRL concentration with cut-off values of 30μIU/ml [43]. Shahin and Raslan carried out the second study in (2007). The purpose of this study was to determine the effectiveness of vaginal fluid BhCG (beta human chorionic gonadotropin), AFP (alpha fetoprotein) and PRL 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. A cut-off value of 20.2 μIU/ml was proposed for PRL and its sensitivity, specificity, positive predictive value, negative predictive value of PRL were 70%, 76%, 71.7%, 74.5% respectively [44]. Kariman N, Hedayati M, Alavi Majd S (2012) carried out the third study in (2012). The sensitivity, specificity, positive and negative predictive values and accuracy were 87.03%, 75.0%, 75.80%, 86.53% and 83.33% respectively in detecting PROM by evaluation of vaginal fluid prolactin concentration with a cut-off value of 9.50 μIU/ml [45]. Results of our study are in good agreement with these three studies. ECLICA method (Electrochemoluminescence assay) was used in Buyukbayrak etal. (2004) [43] and Shahin etal. (2006) [44] to measure PRL that is more sensitive than ELISA. However, it is a costy and complicated test. Also, it is not available in all laboratories. Nevertheless Huber et al. (1993) measured the amount of PRL, AFP and hPL (human placental lactogen) in vaginal washing fluid. The measurement of these proteins in vaginal fluid could not be a suitable clinical test for the diagnosis of PROM even that there are high concentration levels of the three markers in PROM group. They speculated that the reason was the presence of overlap between groups and a high rate of false-positives [46].

In our study, a cut-off value of 500 pg/ml was proposed for ceruloplasmin and its sensitivity, specificity, positive predictive value, negative predictive value of PRL were 85%, 95%, 94.4%, 86.4% respectively. Our study used human Ceruloplasmin ELISA kit (biological fluids) as a method to detect Ceruloplasmin in vaginal wash. One previous study, related to PROM and vaginal washing fluid ceruloplasmin, has been published; this study which was conducted, by Ogino M, Hiyamuta S, Takatsuji-Okawa M, Tomooka Y, and Minoura S (2005), using an original enzyme-linked immune- absorbent assay (ELISA) method that they established, found active ceruloplasmin in human serum [47]. In this study, a cut-off value of 1420.0 ng/mL was proposed for active ceruloplasmin and its sensitivity, specificity, positive predictive value, and negative predictive value of active ceruloplasmin were 85%, 95%, 94.4%, and 86.4% respectively [47]. The prediction method that we established in the present study, if applicable for the prediction of preterm PROM as suggested by Varner (1999) may be helpful for improving perinatal care. Although the time needed for the ELISA assay is usually within 2 hrs, not only time but also cost would be reduced when easy assay kit is developed [48].

In our study, a cut-off value of 2031 μg/L was proposed for Lactate and its sensitivity, specificity, positive predictive value, negative predictive value of Lactate were 95%, 92.5%, 92.7%, 94.9% respectively. Our study used human lactate ELISA kit (biological fluids) as a method to detect lactate in vaginal wash. One previous study was done to establish a relationship between vaginal fluid lactate vaginal fluid and PROM and this was the first time in which lactate concentration determination in vaginal fluids has been used as a diagnostic tool of PROM. This study was conducted by Eva Wiberg-Itzel, Sven Cnattingius, and Lennart Nordstrom (2005). This study used the commercially available Lactate Pro, an electrochemical test strip method, which needs only 5μl of fluids for the

analysis. The test is carried out at the bedside and the result is available after 60 seconds. The meter measures lactate concentrations between 0.8 and 20 mmol/L (proposed name the Lac test). The results of this study showed, a vaginal fluid lactate concentration of 4.5 mmol/L and its sensitivity, specificity, positive predictive value, negative predictive value of were 85%, 91%, 90%, 86% respectively [49].

So, the present study revealed that the diagnostic power of prolactin, ceruloplasmin and lactate was in an acceptable range.

Conclusion

Measurement of vaginal wash Prolactin, Lactate and Ceruloplasmin with ELISA method is a reliable, and non-invasive test for diagnosis of PROM. ELISA, shows better sensitivity, and specificity in measurement of vaginal wash Lactate than Lac test, however Lac test takes shorter time. Measurement of vaginal wash Lactate levels is more sensitive than both Prolactin and Ceruloplasmin. While the specificity of both Prolactin and Ceruloplasmin in vaginal wash are the same and higher than that of Lactate.

Conflict of interest: None. **Disclaimer:** Nil.

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