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Original Research Paper

EVALUATING THE ROLE OF VAGINAL WASH CERULOPLASMIN, LACTATE, AND PROLACTIN IN THE DIAGNOSIS OF PROM

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ABSTRACT

Background: An association has been reported in PROM development and ceruloplasmin in cervicovaginal fluid of females from third-trimester and it is considered that findings on this issue needs further exploration to confirm the role of inflammation in PROM.

Aim: The present study aimed to assess the efficacy of ceruloplasmin, lactate, and prolactin in vaginal washing fluid as a non-invasive and diagnostic marker for PROM (premature rupture of membrane).

Methods: The present study assessed 160 females who presented with PROM to the Institute within the defined study period. These females were randomly selected and underwent a speculum test, sample collection, and atrazine paper test. Vaginal washing fluid samples were assessed for lactate, ceruloplasmin, and prolactin levels using ELISA (enzyme-linked immunosorbent assay).

Results: The study results showed that measuring the lactate levels in vaginal wash fluid samples has high sensitivity compared to ceruloplasmin and Prolactin. However, the specificity of both ceruloplasmin and prolactin in vaginal wash is similar and higher compared to lactate.

Conclusion: The present study concludes that measuring the vaginal wash levels of ceruloplasmin, lactate, and prolactin using enzyme-linked immunosorbent assay method is a non-invasive and reliable test for diagnosis of PROM (premature rupture of membrane).

Keywords: Amniotic fluid, ceruloplasmin, ELISA, lactate, Markers, prolactin, PROM.

INTRODUCTION

PROM (premature rupture of the membrane is fetal membrane rupture before labor onset at any gestational age and is linked to increased ascending infection risk. PROM is either pre-labor or preterm PROM when seen before labor onset or before 37 weeks of gestation. IT can be attributed to various factors. It is a normal process when seen at term, delivery, or labor preparation. However, for preterm, before 37 weeks, it can be problematic.[1]

It can result from weakened membranes from apoptosis or improper collagen arrangement. Previous literature studies report that bacteria in the amniotic fluid of one-third of PROM cases are responsible. In infection response, the body releases inflammatory cytokines that weaken the fetal membrane and increase rupture risk. The fetal membrane acts as a barrier for ascending infection. After membrane rupture, both fetus and mother are at infection risk and increased complications risk. Fetal risk is linked to gestational age primarily. Preterm PROM is linked to 3 times neonatal morbidity increase

including neonatal death, intraventricular hemorrhage, poly microbial intra-amniotic infection, and respiratory distress syndrome, and 4 times increased perinatal morbidity.[2]

Cases are usually seen before labor onset with membrane leak and in a few cases diagnosed easily by vaginal examination. However, in a few cases, it is not diagnosed easily when os is closed or in open os when a membrane bag is present with possible small higher membrane rent. In such situations, laboratory diagnosis is vital including the Fern test and Nitrazine test.[3]

However, low or intermittent volume vaginal discharge, semen, or urine presence may lead to false negative or positive results. Various ancillary techniques to confirm membrane rupture are suggested including some non-specific tests using markers showing decidual disruption rather than membrane rupture. Various markers studies include PAMG 1 (placental alpha microglobulin 1), lactate, urea, creatinine, beta subunit of human chorionic gonadotropin (β hCG), prolactin, alpha-fetoprotein (AFP), and insulin-like growth factor binding protein 1 (IGFBP 1). Also, ultrasonography assessment of oligohydramnios developed after membrane rupture helps in management and diagnosis.[4]

A few invasive tests to diagnose PROM are AF, Amnioscopy for direct visualization of the membranes, and Transabdominal injection of dye (indigo carmine, Evans blue, fluorescein) into the amniotic cavity. However, the single ideal test is not yet developed which should be non-invasive, inexpensive, rapid, and simple. Accurately, the test should not be affected by the presence of contaminants, infected urine, semen, or blood. An appropriate biochemical marker of PROM must have high amniotic fluid concentration, low maternal blood concentration, and extremely low background concentration in cervicovaginal discharge with membrane intact.[5]

Ceruloplasmin is a known antioxidant in plasma which increases in concentration during inflammation. An association has been reported in Ceruloplasmin in cervicovaginal secretions of third-trimester pregnant women and PROM and this finding further confirms the hypothesis that inflammation has a vital role in PROM.[6] Hence, the present study aimed to assess the efficacy of ceruloplasmin, lactate, and prolactin in vaginal washing fluid as a non-invasive and diagnostic marker for PROM (premature rupture of membrane).

MATERIALS AND METHODS

The present study aimed to assess the efficacy of ceruloplasmin, lactate, and prolactin in vaginal washing fluid as a non-invasive and diagnostic marker for PROM (premature rupture of membrane). The study subjects were from the Outpatient Department of the Institute. Verbal and written informed consent were taken from all the subjects before study participation.

The study included 160 pregnant females that visited the Institute within the defined study period and reported vaginal fluid leakage in the third trimester. All subjects underwent sterile Cusco speculum examination for detection of amniotic fluid leakage and allowing congenital anomalies, placental site, amniotic fluid index (AFI), transabdominal sonography to detect fetal viability gestational age (GA), assessment of cervical dilatation, and vaginal fluid sample collection. The inclusion criteria were singleton pregnancy and gestational age of 28-40 weeks from LMP or following 1st-trimester pregnancy. The exclusion criteria were fetal congenital anomalies, medical complications that justify termination of pregnancy such as preeclampsia and diabetes, placenta previa, uterine contraction, and vaginal bleeding.

Included females were categorized following clinical examination and atrazine test results into two groups 80 subjects in Group I with confirmed PROM with positive nitrazine paper test with decreased AFI <10, positive fluid leak upon sterile Cusco speculum examination, and history of watery fluid

leakage and 80 females in Group II with same gestational age with no clinical fluid leakage evidence and negative nitrazine test. Sample collection was done with subjects lying in lithotomy position speculum test and nitrazine test was done with swab to attain a posterior fornix sample. A swab was drawn on nitrazine paper strip. A pH of >6.5 was taken as membrane rupture. Irrigation of the posterior fornix was done with saline. With the same syringe, vaginal wash fluid was aspirated and immediately sent to a laboratory to determine lactate, ceruloplasmin, and prolactin levels by ELISA.

Results were then calculated for lactate, ceruloplasmin, and prolactin levels. Data gathered were statistically analyzed using the chi-square test, Fisher's exact test, Mann Whitney U test, and SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk. NY, USA) using ANOVA, chi-square test, and student's t-test. The significance level was considered at a p-value of <0.05.

RESULTS

The present study aimed to assess the efficacy of ceruloplasmin, lactate, and prolactin in vaginal washing fluid as a non-invasive and diagnostic marker for PROM (premature rupture of membrane). The present study assessed 160 females who presented with PROM to the Institute within the defined study period. AFI (amniotic fluid index) was significantly higher in controls compared to cases with p <0.001. However, the difference in cases and controls was statistically non-significant for abortion, parity, gravidity, gestational age, and age with p=0.741, 0.181, 0.269, 0.126, and 0.345 (Table 1).

It was seen that for Vaginal wash levels of lactate (µg/L), ceruloplasmin (pg/ml), and prolactin (ng/l) in two groups of study subjects, lactate levels were significantly higher in cases compared to controls with p<0.001. Similar significantly higher were seen for ceruloplasmin and prolactin in the case group compared to controls with p<0.001 (Table 2).

The study results showed that for agreement of lactate, ceruloplasmin, and prolactin in the study, a significant agreement was seen in lactate level with p<0.001 with sensitivity, specificity, NPV, and PPV of 95%, 92.3%, 94.7, and 92.5 respectively. Ceruloplasmin levels also showed significant results with p<0.001 with sensitivity, specificity, NPV, and PPV of 85%, 95%, 86.2, and 94.2 respectively. For prolactin, a significant agreement was seen with p<0.001 and with sensitivity, specificity, NPV, and PPV of 70%, 95%, 76, and 93.1 respectively (Table 3).

On assessing the correlation of lactate, ceruloplasmin, and prolactin in study subjects, a significant correlation was seen for prolactin vs ceruloplasmin in cases and controls with p<0.001. A similar significant correlation was seen for lactate vs ceruloplasmin and lactate vs prolactin among cases and controls with p<0.001 (Table 4).

DISCUSSION

The present study assessed 160 females who presented with PROM to the Institute within the defined study period. AFI (amniotic fluid index) was significantly higher in controls compared to cases with p <0.001. However, the difference in cases and controls was statistically non-significant for abortion, parity, gravidity, gestational age, and age with p=0.741, 0.181, 0.269, 0.126, and 0.345. These data were comparable to the previous studies of Wiberg Itzel E et al[7] in 2005 and Ogino M et al[8] in 2005 where authors assessed subjects with demographic and disease data with PROM reported by the authors was comparable to the present study.

The study results showed that for Vaginal wash levels of lactate (µg/L), ceruloplasmin (pg/ml), and prolactin (ng/l) in two groups of study subjects, lactate levels were significantly higher in cases compared to controls with p<0.001. Similar significantly higher were seen for ceruloplasmin and

prolactin in the case group compared to controls with p<0.001. These results were consistent with the findings of Huber JF et al[9] in 1993 and Kariman N et al[10] in 2012 where Vaginal wash levels of lactate (µg/L), ceruloplasmin (pg/ml), and prolactin (ng/l) comparable to the present study was also reported by the authors in their respective studies.

It was seen that for agreement of lactate, ceruloplasmin, and prolactin in the study, a significant agreement was seen in lactate level with p<0.001 with sensitivity, specificity, NPV, and PPV of 95%, 92.3%, 94.7, and 92.5 respectively. Ceruloplasmin levels also showed significant results with p<0.001 with sensitivity, specificity, NPV, and PPV of 85%, 95%, 86.2, and 94.2 respectively. For prolactin, a significant agreement was seen with p<0.001 and with sensitivity, specificity, NPV, and PPV of 70%, 95%, 76, and 93.1 respectively. These findings were in agreement with the results of Shahin M et al[11] in 2006 and Buyukbayrak EE et al[12] in 2004 where the agreement of lactate, ceruloplasmin, and prolactin in vaginal wash reported by the authors in their studies was comparable to the results of the present study.

Concerning the assessment of the correlation of lactate, ceruloplasmin, and prolactin in study subjects, a significant correlation was seen for prolactin vs ceruloplasmin in cases and controls with p<0.001. A similar significant correlation was seen for lactate vs ceruloplasmin and lactate vs prolactin among cases and controls with p<0.001. These results correlated with the findings of Park JS et al[13] in 2007 and Kariman N et al[14] in 2011 where the assessment of the correlation of lactate, ceruloplasmin, and prolactin comparable to the present study was also reported by the authors in their studies.

CONCLUSION

The present study, within its limitations, concludes that measuring the vaginal wash levels of ceruloplasmin, lactate, and prolactin using enzyme-linked immunosorbent assay method is a non-invasive and reliable test for diagnosis of PROM (premature rupture of membrane).

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S. No	Parameters	Cases (n=80)	Controls (n-80)	p-value
1.	**AFI (cm)**	6.06±1.33	15.03±1.89	**<0.001**
2.	**Abortions**	1.06±0.81	1.18±1.05	0.741
3.	**Parity**	0.96±0.84	1.31±1.10	0.181
4.	**Gravidity**	2.13±0.87	2.38±0.94	0.269
5.	**Gestational age (weeks)**	34.63±2.77	35.56±2.56	0.126
6.	**Age (years)**	31.41±3.71	32.36±5.11	0.345

Table 1: Demographics and disease data in two groups of study subjects

S. No	Parameters	Cases (n=80)	Controls (n-80)	p-value
1.	**Lactate**	2462.71±420.07	1751.21±267.04	**<0.001**
2.	**Ceruloplasmin**	571±76.69	457.97±86.92	**<0.001**
3.	**Prolactin**	721.97±109.23	586.13±63.95	**<0.001**

Table 2: Vaginal wash levels of lactate (µg/L), ceruloplasmin (pg/ml), and prolactin (ng/l) in two groups of study subjects

S. No	Parameter	Youden index	Cut-off	p-value	Sensitivity	Specificity	NPV	PPV
1.	**Lactate**	0.873	2029	**<0.001**	95	92.3	94.7	92.5
2.	**Ceruloplasmin**	0.800	500	**<0.001**	85	95	86.2	94.2
3.	**Prolactin**	0.648	657	**<0.001**	70	95	76	93.1

Table 3: Agreement of lactate, ceruloplasmin, and prolactin in the study

S. No		Cases		Control		Total	
		r	p	r	p	r	p
1.	Prolactin vs ceruloplasmin	0.289	0.067	0.083	0.602	0.480	**<0.001**
2.	Lactate vs ceruloplasmin	-0.011	0.0945	0.041	0.789	0.420	**<0.001**
3.	Lactate vs prolactin	0.291	0.065	-0.010	0.938	0.550	**<0.001**

Table 4: Correlation of lactate, ceruloplasmin, and prolactin in study subjects