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Detection of Creatinine in Vaginal Fluid for Diagnosis of Preterm Premature Rupture of Membranes

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ABSTRACT
Background: to evaluate the role of vaginal fluid creatinine level in diagnosis of preterm prelabour rupture of membranes among patients giving history of amniotic fluid leak.
Objectives: the aim of this study was to evaluate the reliability of vaginal fluid creatinine level in the vaginal fluid for diagnosis of prelabour premature rupture of membranes.
Patients and Methods: 100 pregnant women with gestational age 24: 37 weeks gestation participated in the present study divided into 2 groups. Group I (confirmed PROM group) & group II (control non-PROM group). All patients underwent a sterile speculum examination for detection of amniotic fluid pooling in the posterior vaginal fornix followed by taking 3ml from vaginal fluid. Creatinine measurement was done using ELISA.
Results: The mean creatinine levels in vaginal fluid in group (I) & group (II) were.70 ± 0.88 and.04 ± 0.18 mIU/mL respectively. The difference was statistically significant (P value < 0.001). With creatinine cut-off value of 0.25 mIU/ml, the sensitivity & specificity in confirming PROM were 72 and 94% respectively.
Conclusion: Creatinine level was significantly higher in pregnant women with definite PROM. Therefore, vaginal fluid creatinine can be used as an easy, rapid, reliable and non-invasive test for confirming the diagnosis of PROM and can be used as an adjunctive test in equivocal cases.
Keywords: Creatinine, preterm prelabour rupture of membranes.

INTRODUCTION
Prelabour rupture of fetal membranes (PROM) refers to rupture of fetal membrane before the onset of labour. While, preterm PROM (PPROM) refers to rupture of fetal membrane before completing 37 weeks gestation [(1)]. The incidence is about 10% of all gestation and about 2-4% of preterm pregnancies, with complications such as infection and preterm birth [(2)]. Definitive diagnosis of PROM is very important because failure of diagnosis can lead to unwanted obstetric complications as chorioamnionitis, cord prolapse and placental abruption [(3)].

About one-third of women with PPROM develop potentially serious infections, such as intra-amniotic infection (chorioamnionitis and funisitis), endometritis, or septicemia [(1)]. The incidence of placental abruption varies among studies (4%-12%) [(4)]. Conservative management of patients was associated with serious complications that occur early in pregnancy such ad retained placenta or postpartum haemorrhage [(2)]. The fetus and neonate are more affected with PPROM related morbidity and mortality than the mother did. Preterm infants are especicifically vulnerable to many problems, such as respiratory distress syndrome, intra-ventricular hemorrhage, periventricular leukomalacia, infection (eg, sepsis, pneumonia, meningitis), and necrotizing enterocolitis. The incidence of these morbidities vary with gestational age and are higher in the setting of chorioamnionitis [(5)].

The risk to the fetus is high when PPROM occurs before the limit of fetal viability. There is significant risk for maldevelopment of the alveolar tree (pulmonary hypoplasia) as well as fetal compression resulting in malformations similar to those in Potter syndrome with prolonged oligohydramnios [(6)].

The management of patients with PROM, regardless of gestational age is still controversial, thus it is important to reach accurate diagnosis by identifying the presence of specific amniotic fluid markers in vaginal environment [(7)]. There are variable methods used to diagnose PROM are based as much on clinical evaluation as on biological tests, which are useful in the cases of clinically asymptomatic patients and/or in the ones with unclear PROM [(8)]. All these tests have advantages and disadvatages, as measurement of fetal fibronectin, accurate but expensive and time-consuming [(9)]. Vaginal urea and creatinine may be helpful in diagnosis of PROM because fetal urine is the most important source of amniotic fluid in the second half of pregnancy. As hypothesized by **Kafali and Oksuzler [(9)].**

AIM OF THE WORK
The aim of this study was to evaluate the reliability of vaginal fluid creatinine for diagnosis of prelabour premature rupture of membranes.

PATIENTS AND METHODS
Patients:
This study was a prospective cross sectional study that was carried out at Al-Hussein Maternity Hospital. It was based on clinical and biochemical parameters. It was performed on a total of 100 pregnant women between completed 24 and 36 weeks of gestation. They were divided into: 50 pregnant women (confirmed

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PROM group) with history of vaginal fluid leakage and in whom diagnosis of PPROM was suspected and confirmed by amniotic fluid pooling in the posterior fornix, while the other 50 pregnant women were negative for amniotic fluid pooling (normal non PROM group) by Cusco speculum examination.

It was approved by the Ethical Committee of Obstetrics & Gynecology, Al-Hussein Maternity Hospital. For all pregnant women included in this study, explanation of the study procedures was done and informed consent was obtained.

Inclusion criteria:

Pregnant women with high risk for PROM as pre-term premature rupture of membranes in a prior pregnancy, past history of preterm labour, direct abdominal trauma, polyhydramnios and multiple pregnancy. From those pregnant women with any of these risk factors for PROM, only pregnant women that reported a constant vaginal fluid leakage or a sensation of wetness within the vagina or the perineum were selected.

Exclusion criteria:

In this study, some pregnant women were excluded if there was pregnancy-induced hypertension or pre-eclampsia, liver or kidney disease, vaginal bleeding or vaginal infection, fetal congenital anomalies or intrauterine fetal death or any conditions that may have an impact on vaginal fluid creatinine concentrations.

All pregnant women included in this study were subjected to:

(I)- Detailed history including personal history as name, address, age, occupation and special habits. History of present pregnancy including a constant vaginal fluid leakage or a sensation of wetness within the vagina or the perineum, excessive enlargement of the abdomen, direct abdominal trauma, persistent headache, blurring of vision, lower abdominal coliky pain, and recurrent causeless painless fresh bleeding. Menstrual history as last menstrual period to calculate gestational age and expected date of delivery. Obstetric history including parity, mode of previous delivery, previous history of PPROM or preterm labour. Contraceptive history including name of the methods and their duration. Pasthistory of liver or kidney disease, urinary incontinence, hypertension, blood transfusion, allergy to drugs, and operations. Family history for consanguinity, congenital fetal malformations, medical disorder (hypertension, diabetes mellitus) and twins.

(II) - Clinical examination including general examination: especially temperature to exclude infection and blood pressure to exclude hypertension, abdominal examination to assess amniotic fluid volume. Sterile Cusco speculum vaginal examination was done to exclude vaginal bleeding or vaginal infection and then vaginal washing fluid sampling.

(III)- Pelvic ultrasound for: Gestational age determination, amniotic fluid index calculation, multiple pregnancy detection and detection of fetal viability and congenital anomalies.

Methods:

On admission, while each patient of the study in the lithotomy position, a sterile Cusco speculum vaginal examination was performed under complete aseptic condition. Amniotic fluid pooling in the posterior vaginal fornix with or without Valsalva maneuver was noted. After giving an informed consent, 5 ml of sterile saline solution was pooled into the posterior vaginal fornix using a sterile syringe, and 3 ml of the pooled saline was aspirated with the same syringe, then sent immediately to laboratory of Biochemistry Department at Al-Hussein Hospital for assay of creatinine (by spectrophotometer 5010 supplied by German Boehringer Mannheim Company) using colorimetric method. The concentration of creatinine was read by spectrophotometer at wave length 492 nm **[10].**

Ultrasonic assessment of amniotic fluid index (AFI) for each subject of the study using four-quadrant technique. The AFI is considered normal between 8.1 and 18, low between 5.1 and 8, very low ≤ 5, high > 18 **[11]**. Subjects included in this study were divided into two groups: confirmed PROM group and normal non PROM group. Subjects who were positive for amniotic fluid pooling in the posterior vaginal fornix during a sterile Cusco speculum vaginal examination, had lower gestational age by fundal level examination than expected by date and had lower AFI by ultrasound at time of obtaining the sample were taken as "confirmed PROM group".

Subjects who were negative for amniotic fluid pooling in the posterior vaginal fornix during a sterile Cusco speculum vaginal examination, had the same gestational age by fundal level examination corresponding to that expected by date and had normal AFI by ultrasound at time of obtaining the sample were taken as "normal non PROM" group.

The parameters of maternal age, parity, gestational age at time of obtaining the sample, mode of delivery, fetal presentation, and vaginal fluid creatinine levels were also documented and compared between both groups by using unpaired t-test for quantitative variables in parametric data and Chi-square test for qualitative variables. Receiver operating characteristic (ROC) curve analysis was used to establish an optimal cut-off concentration. The results were evaluated with a significance level of $P < 0.05$.

Statistical methodology

Data were coded and entered using the statistical package SPSS version 23. Data were summarized

using mean, standard deviation, median, minimum and maximum for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test [12]. For comparing categorical data. Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [12]. ROC curve was constructed with area under curve analysis performed to detect best cutoff value of creatinine for detection of PROM. P-values less than 0.05 were considered as statistically significant.

RESULTS

Table (1): Comparison between the two groups regarding maternal age:

	PROM cases					non PROM CASES					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Age (years)	25.90	5.33	26.00	18.00	39.00	27.56	5.87	27.00	17.00	41.00	0.142

The age of the patients ranged between 17 and 41 years with a mean of 25.90 ± 5.33 and 27.56 ± 5.87 years in Group (I) & (II) respectively. No statistically significant difference between the 2 groups regarding maternal age (P value 0.142).

Table (2): Comparison between the two groups concerning gestational age:

	PROM cases					non PROM CASES					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
A (weeks)	34.06	1.41	35.00	30.00	36.00	33.42	2.89	34.00	28.00	36.00	0.279

The mean GA in Group (I) & (II) were 34.06 ± 1.41 and 33.42 ± 2.89 weeks respectively, with no statistically significant difference between the 2 groups as regard GA (P value = 0.279)

Table (3): Comparison between the two groups according to parity:

		PROM cases		non PROM CASES		P value
		Count	%	Count	%	
Parity	Para 0	0	.0%	1	2.0%	0.298
	Para 1	8	16.0%	10	20.0%	
	Para 2	14	28.0%	21	42.0%	
	Para 3	6	12.0%	5	10.0%	
	Para 4	2	4.0%	0	.0%	
	Para 6	2	4.0%	0	.0%	
	PG	18	36.0%	13	26.0%	

The percentage of patients in group (I) with para 0, 1, 2, 3, 4, 5, 6, PG was 0, 16.0, 28.0, 12.0, 4.0, 4.0, 36.0 % respectively compared to the percentage of patients in group (II) with para 0, 1, 2, 3, 4, 5, 6, PG was 2.0, 20.0, 42.0, 10.0, 0, 0, 26.0 % respectively. No statistically significant difference between the 2 groups as regard parity (P value = 0.298).

Table (4): Comparison between the two groups according to mode of delivery:

		PROM cases		non PROM CASES		P value
		Count	%	Count	%	
ode of delivery	NVD	21	72.4%	15	41.7%	0.095
	3CS	1	3.4%	4	11.1%	
	2CS	1	3.4%	3	8.3%	
	1CS	6	20.7%	14	38.9%	

The percentage of patients in group (I) with NVD, 3CS, 2CS,1CS was 72.4, 3.4, 3.4 & 20.7% respectively compared to the percentage of patients in group (II) with NVD, 3CS, 2CS, 1CS was 41.7, 11.1, 8.3 & 38.9% respectively. No statistically significant difference between the 2 groups as regards mode of delivery (P value = 0.095).

Table (5): Amniotic fluid index (AFI) among the two groups:

	PROM cases					non PROM CASES					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
AFI (cm)	4.30	1.64	4.50	1.00	8.00	11.60	2.60	11.50	8.00	17.00	< 0.001

The mean AFI was 4.30 ± 1.64 cm in group (I). On the other hand, the mean AFI was 11.60 ± 2.60 cm in group (II) respectively, with high significant difference between the 2 groups as regard AFI (P value < 0.001).

Table (6): Comparison between the two groups according to fetal presentation:

		PROM cases		non PROM CASES		P value
		Count	%	Count	%	
Fetal presentation	Transverse	0	.0%	1	2.0%	0.012
	Cephalic	47	94.0%	37	74.0%	
	Breech	3	6.0%	12	24.0%	

The percentage of patients in group (I) with transverse lie, cephalic & breech presentations was 0, 94.0 & 6.0 % respectively compared to the percentage of patients in group (II) with transverse lie, cephalic & breech presentations was 2.0, 74.0 & 24.0% respectively. There was no statistically significant difference between the 2 groups as regard fetal presentation (P value 0.012).

Table (7): Creatinine level in the vaginal fluid among the two groups:

	PROM cases			non PROM CASES			P value
	Mean	SD	Median	Mean	SD	Median	
Creatinine mg/dl	0.70	0.16	0.50	0.04	0.08	0.00	< 0.001

The mean vaginal fluid creatinine levels in Group (I) & Group (II) were .70 ± .88 & .04 ± .18 mIU/mL respectively. The difference was statistically significant (P value < 0.001).

Table (8): Sensitivity, specificity, lower bound, upper bound & cutoff value of creatinine levels ≥ 0.25 mIU/mL in diagnosis of PROM.

Area under curve	P value	95% Confidence Interval		Cutoff value	ensitivity (%)	pecificity (%)
		Lower Bound	Upper Bound			
.838	<0.001	.755	.922	0.25	72	94

With creatinine cutoff value of 0.25 mIU/mL, the Sensitivity & specificity in diagnosis of PROM were 72 and 94 % respectively.

DISCUSSION

The current study was done to evaluate the reliability of vaginal fluid creatinine in the diagnosis of premature rupture of membranes.

Kafali and Oksuzler [9] hypothesized that vaginal urea and creatinine may be useful in the diagnosis of PROM as fetal urine is the most important source of amniotic fluid in the second half of pregnancy and they proved that in 2007.

This study showed that the mean vaginal fluid creatinine levels in definite PROM and control groups using unpaired t test were.70 ± .88 mIU/ml and 0.04 ± 0.18 mIU/ml, respectively, and the difference was highly statistically significant (p value < 0.001) as shown in table (7).

Creatinine level sensitivity and specificity were 72% and 94% respectively with a cut off value of 0.25 mg/ dl (table 8).

These results go with that of the study performed by **Ghasemi *et al.*** [13] who found that creatinine was 0.86 ± 0.68 mg/dL, in the investigation group and 0.20 ± 0.16 mg/dL in the control group. The results were significant (p < 0.001). Based on the receiver operating characteristic curve the cut-off point for creatinine was 0.25 mg/dL and it had 74.6% sensitivity, 85% specificity, and 83% and 77.2% positive and negative predictive values for diagnosis of PROM.

These results are comparable with the study performed **Tigga and Malik** [14] who found that vaginal washing concentration of creatinine was significantly higher in the study group (p < 0.01) with

mean vaginal fluid creatinine levels in confirmed PROM group and control group were 0.26 ± 0.0663 versus 0.09. ± 0.0414 mg/ml respectively. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for creatinine were 100%, 92%, 92.59%, 100% and 96% respectively.

Also, these results are found to be comparable with the study performed by **Urdaneta *et al.*** [15] who found that mean vaginal fluid creatinine levels in confirmed PROM group and control group were 1.09 ± 0.35 mg/dl and 0.36 ± 0.17 mg/dl respectively. The difference was statistically significant (p-value < 0.05) with sensitivity, specificity, positive predictivity, and negative predictivity were 78.3%, 78.7%, 80.6% and 76.4% respectively and cut off value of 0.45 mg/dl.

These results cope with the study performed by **Zanjani and Haghighil** [16] who found that the mean vaginal fluid creatinine levels in confirmed PROM group, suspected PROM group and control group were 1.74 ± 0.8 mg/dl, 0.45 ± 0.2 and 0.25 ± 0.1 mg/dl respectively. The difference was statistically significant (p-value < 0.001) with sensitivity, specificity, positive predictivity, and negative predictivity were 96.7%, 100%, 100% and 96.8% respectively and cut off value of 0.5 mg/dl.

Also, these results cope with the study performed by **Tavana *et al.*** [17] who found that the mean vaginal fluid creatinine levels of confirmed PROM group, suspected PROM group and control group were 0.22 ± 0.08, 0.55 ± 0.04 and 0.07 ± 0.02 mg/dl respectively. The difference was statistically significant (p < 0.05). The sensitivity, specificity and positive and negative predictive values were 90.2%, 91.2%, 83.6% and 90% respectively in detecting PROM by evaluation of vaginal fluid creatinine concentration with a cut off value of 0.75mg/dl.

In study done by **Kafali and Oksuzler** [9], it was found that mean vaginal fluid creatinine levels in definite, suspected and control groups were 1.5 ± 0.3 mg/dl, 0.34 ± 0.22 mg/dl and 0.28 ± 0.23 mg/dl respectively. The difference was statistically significant (p-value < 0.01) with sensitivity, specificity, positive predictivity, and negative predictivity were 100%, 100%, 100% and 100% respectively and cut off value of 0.6 mg/dl. In addition, these results cope with results of **Li and Chang** [18] where sensitivity, specificity, positive predictivity, and negative predictivity were 94%, 90%, 100% and 88.6% respectively in detecting PROM by evaluation of vaginal fluid creatinine concentration with cut off value of 0.55 mg/dl.

Gurbuz *et al.* [19] reported that the sensitivity specificity, positive predictivity, and negative predictivity were all 100% in detecting PROM by evaluation of vaginal fluid creatinine concentration.

Urdaneta *et al.* [15] reported that determination of vaginal fluid creatinine concentrations was a useful diagnostic tool for premature rupture of membranes. Besides, **Zanjani and Haghighil** [16] reported that vaginal fluid creatinine determination for diagnosis of premature rupture of membranes was a reliable, simple, rapid and inexpensive. Moreover, **Kafali and Oksuzler** [9] have found that vaginal washing fluid urea and creatinine determination for the diagnosis of PROM was a reliable, simple and rapid test. In addition, **Gurbuz *et al.*** [19] showed that vaginal fluid creatinine was an extremely useful marker in doubtful cases of PROM. In these cases, new methods such as αFP, HCG and fetal fibronectin were investigated. However, they have low specificity owing to overlap between the values of αFP, HCG, and fibronectin in patients with and without intact membranes [19].

This study showed that creatinine assay is cheap as it costs 15 pounds and fast method, as it takes 20 minutes and has high sensitivity and specificity to establish accurate diagnosis. Thus, it is a possible candidate to become a gold standard test for diagnosis of cases of PROM as it is cheaper, faster, higher sensitivity and specificity than α-FP, β-HCG and fetal fibronectin.

CONCLUSION

Vaginal fluid creatinine determination for the diagnosis of PPROM is a rapid, simple, and non-invasive method and had higher sensitivity and specificity to establish accurate diagnosis. It is a possible candidate to become a gold standard diagnostic test for PROM.

REFERENCES

1. **Patrick D, Charles JL, Vanessa AB (2012):** Preterm premature rupture of membranes. Am J Obstet Gynecol., 212: 453-55.
2. **Modena AB, Kaihura C, Fieni S (2004):** Prelabour rupture of the membranes: recent evidence. Acta Biomed Ateneo Parmense., 75 (2): 9-12.
3. **Kim VM, Park KH, Chaworrapongsa SA (2005):** Matrix metalfoproteinase3 in parturition. Premature rupture of membrane and microbial invasion of the amniotic cavity. J Pernat Med., 31 (1):12-22.
4. **Mercer BM, Moretti M, Prevost RR (1992):** Erythromycin therapy in preterm rupture of the membranes: a prospective trial of 220 patients. Am J Obstet Gynecol., 166: 794-802.
5. **Soraisham AS, Singhal N, McMillan DD (2009):** A multicenter study on the clinical outcome of chorioamnionitis in preterm infants. Am J Obstet Gynecol., 200: 372.
6. **DeFranco E, Atkins K, Heyl P (2007):** Preterm labor, premature rupture of membranes and cervical insufficiency. Manual Obstetrics, 7th Ed., Lippincott Williams & Wilkins.
7. **Chen FCK and Dudenhausen JW (2008):** Comparison of two rapid strip tests based on IGFBP-1 and PAMG-1 for the detection of amniotic fluid. American Journal of Perinatology, 25 (04): 243-6.
8. **Modena AB, Rabello YA (2007):** Diagnosis of premature rupture of fetal membranes. Am J Obstet Gynecol., 196: 438.

9. **Kafali H, Oksuzler C (2007):** Vaginal fluid urea and creatinine in diagnosis of premature rupture of membranes. Arch Gynecol Obestet., 275 (3): 157-160.
10. **Murray RL (1984):** Clinical Laboratory Tests. Clin Chem the C.V. Mosby Co. St Louis. Tornoto. Princeton.
11. **Phelan JP, Ahn MO, Smith CV *et al.* (1987):** Amniotic fluid index measurements during pregnancy. J Reprod Med., 32: 601-604.
12. **Chan YH (2003):** Biostatistics 102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J., 44 (8): 391-396.
13. **Ghasemi M, Jaami R, Alleyassin A *et al.* (2016):** The value of urea, creatinine, prolactin, and beta sub-unit of human chorionic gonadotropin of vaginal fluid in the diagnosis of premature preterm rupture of membranes in pregnancy. Turk J Obstet Gynecol., 13: 62-66.
14. **Tigga M, Malik S (2015):** Comparative analysis of four biomarkers in diagnosing premature rupture of membranes and their correlation with onset of labour. Int J Reprod Contracept Obstet Gynecol., 4 (4): 1070-1075
15. **Urdaneta G, Reyna V, Torres C, Santos B (2013):** Determination of vaginal fluid creatinine concentrations in premature rupture of membranes. Eur J Obstet Gynecol Reprod Biol., 171: 156-162.
16. **Zanjani MS, Haghighil KM (2012):** Vaginal fluid creatinine for detection of PROM. Japan Society of Obstetrics and Gynecology. J Obstet Gynecol Res., 38 (3): 505-508.
17. **Tavana Z, Hamedi B, Zolghardi J, Madaadi G (2010):** Evaluation of diagnostic role of vaginal fluid urea, creatinine and B-HCG level for detection of PROM. Internet Journal of Gynecology and Obstetrics, 15: 1.
18. **Li HY, Chang TS (2000):** Vaginal fluid creatinine, human chorionic gonadotropin and alpha-fetoprotein for detection of premature rupture of membranes. Am J Obstet Gynecol., 63: 686-690.
19. **Gurbuz A, Karateke A, Kabaca C (2004):** Vaginal fluid creatinine in premature rupture of membranes. Int J Gynecol Obstet., 85: 270-271.