Vaginal fluid creatinine for the detection of pre-labor rupture of membranes*

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ABSTRACT

Introduction: Prelabor rupture of membranes (PROM) occurs in 8% of all pregnancies and is a major cause of preterm birth and perinatal morbidity and mortality. In 47% of cases, clinicians are uncertain regarding the diagnosis of PROM based on examination and history alone. A misdiagnosis often leads to unnecessary interventions that may be detrimental to mother and fetus. There is currently no ideal noninvasive diagnostic test that can diagnose prelabor rupture of membranes with certainty.

Objective: This study aims to determine if a quantitative assay of vaginal fluid creatinine can correctly diagnose prelabor rupture of membranes in women with singleton pregnancies at 28-42 weeks age of gestation

Methodology: A prospective study was performed at a tertiary hospital from December 2015 to August 2017 with a computed sample size of 180 patients (60 per group). If a history of watery discharge was confirmed by egress of fluid, then the patient was included in the Ruptured membranes group. If despite a history of watery discharge, no egress is noted, then she was included in the Unsure membrane status group. 60 women with normal pregnancies were randomly chosen for the control group. Vaginal fluid was collected for Litmus Paper, Fern, and Vaginal Fluid Creatinine Tests.

Results and Conclusion: Vaginal fluid creatinine at 1.00 mg/dL has higher sensitivity, specificity, positive and negative predictive values, and a higher positive likelihood ratio than the litmus paper or ferning tests. High accuracy values, with a low false negative rate of 0, and a large AUC make vaginal fluid creatinine an excellent test for the detection of PROM, in accordance with previous studies.

Keywords: Prelabor rupture of membranes, PROM, creatinine, PPROM, preterm prelabor rupture of membranes, amniotic fluid, litmus paper test, nitrazine test, ferning

INTRODUCTION

Prelabor rupture of membranes (PROM) occurs in 8% of all pregnancies and is a major cause of preterm birth and perinatal morbidity and mortality.[1] While the diagnosis of prelabor rupture of membranes may be simple and outright in cases of obvious egress of amniotic fluid, the diagnosis becomes more elusive in cases where a history of watery vaginal discharge is not followed by a speculum exam finding of pooling or egress of fluid. Recent data suggest that in 47% of cases, clinicians are uncertain regarding the diagnosis of PPROM based on clinical examination by sterile speculum examination and patient history alone.[2] A misdiagnosis often leads to a cascade of inappropriate or unnecessary interventions that may be detrimental to both mother and fetus.

The optimal approach to the diagnosis and management of PROM remains controversial. While common methods like the litmus paper and fern test are readily available, they have a low sensitivity and specificity. More accurate methods that detect proteins in amniotic fluid like fetal fibronectin (Quick Check fFN test®), placental alpha microglobulin-1 (PAMG-1 – AmniSure®), and insulin growth factor binding protein-1 (IGFBP-1: Actim® PROM) may not be readily available in small centers and they may be costly. The more definitive test for the diagnosis of PROM, the

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amnio-dye test, is invasive, and carries risks of infection and abruption. Other tests developed include amniotic fluid alpha fetoprotein, human chorionic gonadotropin, diamine oxidase, prolactin, urea, creatinine, aspartate aminotransferase and alanine aminotransferase. There is currently no ideal noninvasive diagnostic test that is simple, reliable, accurate, readily available, low-cost, and fast, that can diagnose prelabor rupture of membranes with certainty.

Given that the production of amniotic fluid is predominantly accomplished by excretion of fetal urine (~300 ml/Kg fetal weight/day or 600 to 1200mL/day near term)[3] and that fetal urine consists primarily of water, creatinine, and urea[1], it has been stipulated that creatinine in vaginal fluid may indicate rupture of membranes, especially since creatinine is not normally found in vaginal fluid. Hence, this study aims to evaluate vaginal fluid creatinine (VFC) for the diagnosis of prelabor rupture of membranes. The results of this study are of potential value for obstetricians, giving them a new tool to aid them in the diagnosis of equivocal cases of PROM.

RESEARCH QUESTION

What is the diagnostic utility of vaginal fluid creatinine in the detection of prelabor rupture of membranes in women with singleton pregnancies at 28-42 weeks age of gestation?

OBJECTIVES

General Objective: To determine if a quantitative assay of vaginal fluid creatinine can correctly diagnose prelabor rupture of membranes in women with singleton pregnancies at 28-42 weeks age of gestation

Specific Objectives: To compare the sensitivity, specificity, positive predictive value, and negative predictive value of vaginal fluid creatinine test, nitrazine or pH or litmus paper test, and ferning test in determining prelabor rupture of membranes

To determine the optimal cut-off value of vaginal fluid creatinine that detects prelabor rupture of membranes

To determine if gestational age has an effect on the amount of creatinine found in vaginal fluid

To determine if latency (length of time from rupture of membranes to speculum exam) has an effect on the amount of creatinine found in vaginal fluid

REVIEW OF RELATED LITERATURE

Prelabor rupture of membranes (PROM) refers to rupture of membranes 1 hour or more before onset of labor[4], and is a part of a continuum of preterm prelabor rupture of membranes (PPROM) - membrane rupture before labor and before 37 weeks age of gestation; and previable prelabor rupture of membranes - rupture of membranes before viability (previable PPROM).

The diagnostic confirmation in ambiguous cases is a major challenge, because correct diagnosis is necessary to decide on the most appropriate management and ultimately to reduce both maternal and fetal complications. Limitations of the accuracy of tests may lead to unnecessary interventions while poor sensitivity may be falsely reassuring and delay or deprive women of appropriate treatments, increasing the risk of potential maternal and fetal morbidity and mortality. Hence, the optimal test should be highly sensitive and specific for amniotic fluid and not be affected by contamination from other body substances or vaginal medications.

Membrane rupture can be diagnosed unequivocally with ultrasonographically-guided transabdominal instillation of indigo carmine dye, followed by the passage of blue-dyed fluid into the vagina, documented by a stained tampon or pad. Of note, maternal urine also will turn blue and should not be confused with amniotic fluid[5].

The diagnosis of membrane rupture typically is confirmed by direct visualization of amniotic fluid passing from the cervical canal and pooling in the vagina (egress); a basic pH test of vaginal fluid; or arborization (ferning) of dried vaginal fluid, identified under microscopic evaluation. Studies have shown that these methods can have sensitivities of 51% to 98% and specificities of about 70-88%. Ferning has been associated with false positive results in 5-30% and false negative results in 5-12.9%. pH, Litmus paper, or the Nitrazine test has been associated with false positive results in 17.4% and false negative results in 12.9%[6]. False-positive test results may occur in the presence of blood or semen, alkaline antiseptics, or bacterial vaginosis. False-negative test results may occur with prolonged membrane rupture and minimal residual fluid[5]. Despite the low accuracy of these tests, they are still used in places where access to more accurate rapid immunoassays are limited, as in many hospitals in the country.

In equivocal cases, additional tests may aid in the diagnosis. Ultrasonographic examination of amniotic fluid volume may be a useful adjunct, but is not diagnostic[5]. The absence of a non-invasive gold standard for the diagnosis of rupture of membranes resulted in the development of several tests based on alternative biochemical markers found in amniotic fluid.

Amniotic fluid has a composition similar to that of the maternal plasma and contains some sloughed fetal cells from the skin, digestive system, and urinary tract; and biochemical substances produced by the fetus, such

Table 1. Number of cases (or controls) for expected sensitivities (or specificities) ranging from 0.91 to 0.99

Expected sensitivity (or specificity)	Minimal acceptable lower confidence limit													
	0.85	0.86	0.87	0.88	0.89	0.9	0.91	0.92	0.93	0.94	0.95	0.96	0.97	0.98
0.91	319	438	666	1,127	2,443	9,309								
0.92	220	294	403	613	1,035	2,215	8,428							
0.93	166	203	273	372	549	934	1,992	7,512						
0.94	126	153	183	248	334	493	832	1,763	6,576					
0.95	93	109	137	169	217	298	434	729	1,524	5,626				
0.96	76	82	98	117	151	191	253	374	625	1,288	4,654			
0.97	59	63	79	85	105	129	158	224	309	519	1,036	3,643		
0.98	50	53	58	63	69	89	115	129	185	259	386	781	2,620	
0.99	50	50	50	51	56	61	68	77	109	127	181	261	521	1,567

The probability that the estimated 95% lower confidence limit is above the minimal acceptable value is 0.95.

as bilirubin, lipids, enzymes, electrolytes, nitrogenous compounds, and proteins[7]. Prolactin and AFP were not useful markers because of the overlap in concentrations between women with and without ruptured membranes[8]. Human chorionic gonadotropin is also not useful because quantitative HCG takes long and is costly[9]. Fetal fibronectin is a sensitive but nonspecific test for ruptured membranes; a negative test result is strongly suggestive of intact membranes, but a positive test result is not diagnostic of PROM[10]. Other tests like insulin-like growth factor binding protein-1 (IGFBP-1: Actim® PROM), placental alpha microglobulin-1 (PAMG-1 – AmniSure) show promise but are expensive and not readily available in many centers in the Philippines. There is currently no single test that is superior to the others in diagnosis[11,12,6].

Anchoring on the fact that fetal urine is the most important source of amniotic fluid in the third trimester[1], studies have suggested that an elevated creatinine in vaginal fluid may be indicative of ruptured membranes from leakage of amniotic fluid into the vagina[13-21].

Creatinine is absent in vaginal fluid except in cases of vesico-vaginal fistula or accidental puncture of the bladder during specimen collection.

In the first half of pregnancy, creatinine concentrations are similar in maternal serum and in amniotic fluid (~0.6 mg/dL). This then increases gradually between 20 to 32 weeks of gestation and more rapidly thereafter, when they are two to four times higher than in maternal serum. Prior to 36 weeks' gestation, the amniotic fluid creatinine level ranges between 1.5 and 2.0 mg/dL[7]. A creatinine concentration of 1.75 mg/dL or more is correlated significantly with a gestational age of 37 weeks or more[22]. This rise in creatinine near term is due to increasing maturation of the fetal renal system as well as increased fetal muscle mass[23]. Creatinine in amniotic fluid can be distinguished from maternal urine in that it does not exceed 3.5 mg/dL, whereas in adult urine, values as high as 100 mg/dL may be found[24]. Creatinine also has the advantage of being low cost and readily available in centers where measurement of other body fluid (serum/urine) creatinine is available. Previous studies on creatinine, however, have not agreed on an optimum cut-off point for the detection of rupture of membranes, with cut-off values ranging from 0.12-1.05mg/dL.

METHODOLOGY

A prospective study was performed at a tertiary hospital from December 2015 to August 2017, among all patients who consented. This study was approved by the hospital's institutional review board last October 30, 2015. A total sample size of 180 patients (60 in each group) was computed based on the approach provided by Flaulhalt, Cadilhac, and Thomas[25]. Table 1 is based on the formula Ncontrols = Ncases [(1-Prev)/Prev] Using data from previous studies, an expected sensitivity of 97% and a 0.85 lower confidence interval was used.

Subjects were recruited into the study after meeting the following criteria: singleton pregnancy at 28–40 weeks age of gestation (calculated according to ultrasound done before 12 weeks gestation), and without these exclusion criteria: imminent delivery, fetal distress, known comorbidities that affect fetal urine production, poly- or oligohydramnios, placenta previa, history of vaginal bleeding or spotting, current vaginal infection, vaginal washing with antiseptics, obvious blood or meconium staining of amniotic fluid, vaginal intercourse in the previous 48 hours, internal exam immediately prior to their evaluation for ROM, or maternal urine admixing with amniotic fluid. Informed consent was obtained. Subjects were informed that they may withdraw from the study at any time without effect on the management of their case.

Demographic and pertinent obstetric history were obtained. The time from rupture of membranes to physical exam was also calculated.

After the interview, urine was collected for quantitative urine creatinine, which was compared with vaginal fluid creatinine (VFC) to ensure that the vaginal fluid was not contaminated by urine during specimen collection. After voiding, sterile speculum exam was done. Patients were examined in dorsal lithotomy position with good illumination using an appropriately-sized sterile speculum lubricated with sterile water. 3mL of sterile water was introduced into the posterior vaginal fornix,

and after 1-2 minutes, up to 5mL was aspirated from the posterior fornix using the same syringe. Care was taken so as not to accidentally contaminate the specimen with maternal urine. 2 drops of fluid was used for the litmus paper test, and another 2 drops for the ferning test. A KOH smear and saline drop test was also performed on another 2 drops of fluid to rule out infection. The remaining 4mL of fluid was centrifuged and sent for quantitative creatinine determination.

While the sensitivity and specificity of the ferning and litmus paper tests are low, they are still included because they are the current standard diagnostic tests used in the country. Creatinine concentration was estimated by the AutoAnalyzer using the automated version of Jaffe's method of picric acid concentration. Internal examination, if necessary, was performed after collection of specimens. All speculum examinations and sample collections were performed by the same physician; all laboratory tests were performed by the same medical technologists who were blinded regarding the membrane status of the patient.

Patients were grouped as follows: If a history of watery vaginal discharge was confirmed by obvious egress of amniotic fluid, then the patient was included in the confirmed ruptured membranes group (Group 1: Ruptured membranes group). However, if despite a history of watery discharge, no egress is noted, regardless of litmus or fern test status, she was then included in the suspected PROM group (Group 2: Unsure membrane status group). 60 women with normal pregnancies consulting at the outpatient clinic for prenatal check up with no history of watery discharge, or any other complaints or complication, or abnormal vaginal discharge, were randomly chosen and assigned to the control group (Group 3: Intact membranes/ control group). Each group had 60 subjects for a total of 180 subjects, as previously computed. (Figure 1)

Statistical analysis using SPSS 25 was performed. Age, parity, gestational age, and vaginal and urine creatinine levels were compared using the Student T-test, and one-way ANOVA. Receiver operating characteristic (ROC) curve analysis was used to establish an optimal cut-off point for vaginal fluid creatinine. The significance level was set at 0.05. Sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratios were also computed for creatinine, as well as the litmus paper and ferning tests. The Pearson correlation coefficient was used to determine the association between vaginal fluid creatinine and age of gestation; and vaginal fluid creatinine and latency.

RESULTS

A total of 205 women were recruited, 22 were disqualified. 183 women were included in this study, with 61 women in each group (Table 2). Table 2 shows the demographic data of the 3 groups. The mean age was 30 years, with a range of 18-45 years. The average gestational age is 37 weeks, with a range of 28-42 2/7 weeks, and the average parity is 2, with a range of 1-7. There was no significant difference in age, gestational age, and parity between the 3 groups.

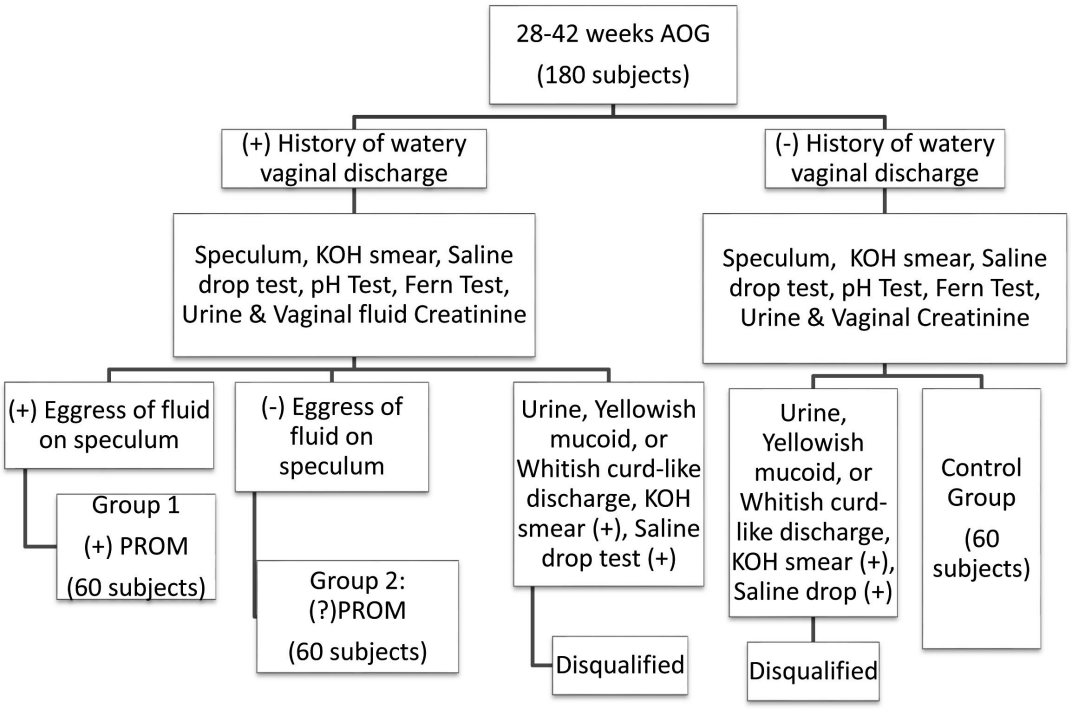

Figure 1. Schematic diagram of study procedure

Table 2. Difference between groups in terms of demographic data

	Group 1 (+) PROM (n=61)	Group 2 (?) PROM (n=61)	Group 3 (-) PROM (n=61)	Mean for all	F*	p*
Age (years)	29 (18-44)	30 (19-43)	30. (18-45)	30. (18-45)	0.627	0.535
Gestational age (weeks)	37 (28 – 40 3/7)	37 (32 2/7 – 41 4/7)	37 (32 2/7 – 42 4/7)	37 (28 – 42 2/7)	0.978	0.378
Parity	2 (1-7)	2 (1-6)	2 (1-6)	2 (1-7)	2.03	0.134

* Analysis using one-way ANOVA test

Majority of patients were service cases, and were high school graduates. 75.4% and 38.8%, respectively; with a p-value of 0.212 meaning there was no significant difference between groups in terms of distribution of private/service cases (Tables 3 and 4, Figures 2 and 3)

Table 3. Subjects' Educational Attainment

	Frequency	Percent
Grade School	4	2.2
High School	71	38.8
Undergrad	51	27.9
College	57	31.1
Total	183	100.0

Table 4. Distribution of subjects in terms of private or service

	Frequency	Valid Percent
Service	138	75.4
Private	45	24.6
Total	183	100.0

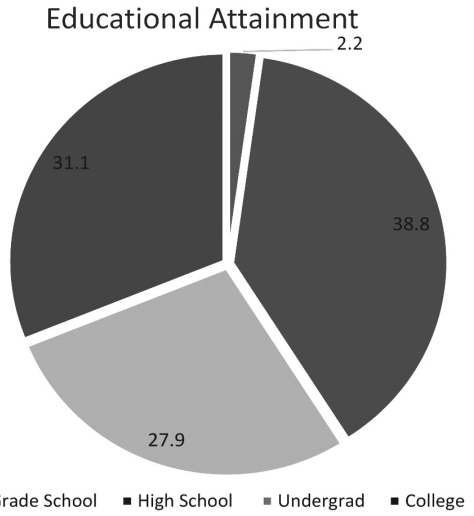

Figure 2. Distribution of subjects in terms of educational attainment

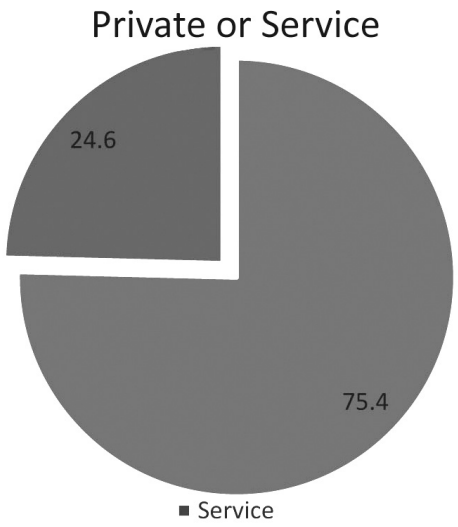

Figure 3. Distribution of subjects in terms of private or service

The mean urine creatinine value is 58.59 mg/dL, while the mean vaginal fluid creatinine value is 0.72 mg/dL. (Table 5) The independent t-test showed that the vaginal and urine creatinine values are significantly different. This means that in all the vaginal fluid samples, there was no contamination from maternal urine (Table 6).

Table 7 shows the means and ranges of the vaginal fluid creatinine results of the 3 groups. Group 1 with confirmed rupture of membranes had the highest mean vaginal fluid creatinine, while the confirmed intact membranes had the lowest mean vaginal fluid creatinine values. The values range from 0-0.3 in this group, indicating that creatinine is almost absent in the vaginal fluid of those with confirmed intact membranes. Meanwhile, the values for the confirmed ruptured membranes group range from 1-3.6, indicating that creatinine is significantly present in the vaginal fluid of those whose membranes are ruptured. Those with unsure membrane status have VFC ranges from 0-2.6, indicating that some members of this group had no creatinine in their vaginal fluid, while others had. The ANOVA test showed that there is a significant difference between the vaginal fluid creatinine values of the 3 groups (Table 7). The independent t-test between groups showed that the values in group 1 are significantly higher than the values in group 2, and group 3, and that group 3 has

Table 5. Descriptive statistics for Vaginal and Urine Creatinine

		N	Mean (mg/dL)	Std. Deviation	Std. Error Mean
Creatinine	Urine	183	58.9597	40.92209	3.02505
	Vaginal	183	.7251	.98370	.07272

Table 6. Independent t-test comparing vaginal and urine creatinine

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
Creatinine	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	199.292	.000	19.245	364	.000	58.23452	3.02592	52.28404	64.18500
Equal variances not assumed			19.245	182.21	.000	58.23452	3.02592	52.26417	64.20487

Table 7. Difference between groups in terms of vaginal fluid creatinine

	Group 1 (n=61)	Group 2 (n=61)	Group 3 (n=61)	F	p
Vaginal fluid creatinine	1.94 (1-3.6)	0.23 (0-2.6)	0.0079 (0-0.3)	304.521	0.000
P	Group 1 vs 2 0.000	Group 1 vs 2 0.000	Group 1 vs 3 0.000		
95% confidence interval	1.49-1.91	0.07-0.36	1.78-2.08		

significantly lower values than the other groups. This is also demonstrated by the boxplot chart (Figure 4).

A Receiver Operator Characteristic (ROC) curve was constructed to determine the optimal cut-off value that will give the most sensitive and specific test. The area under the curve is 0.98, greater than 0.9, indicating that vaginal fluid creatinine is an excellent test for confirming rupture of membranes (Figure 5 and Table 8)

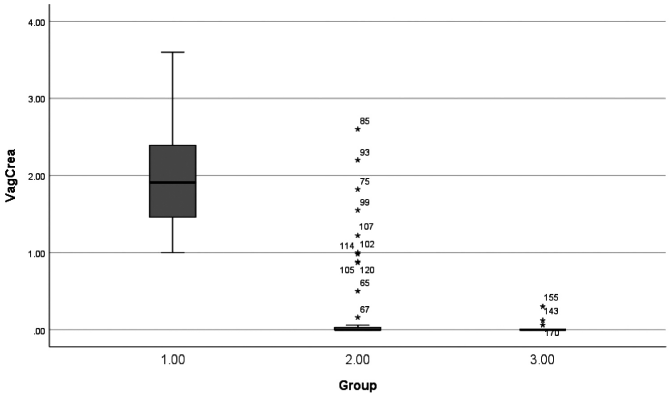

Figure 4. Boxplot chart for vaginal fluid creatine among the 3 groups

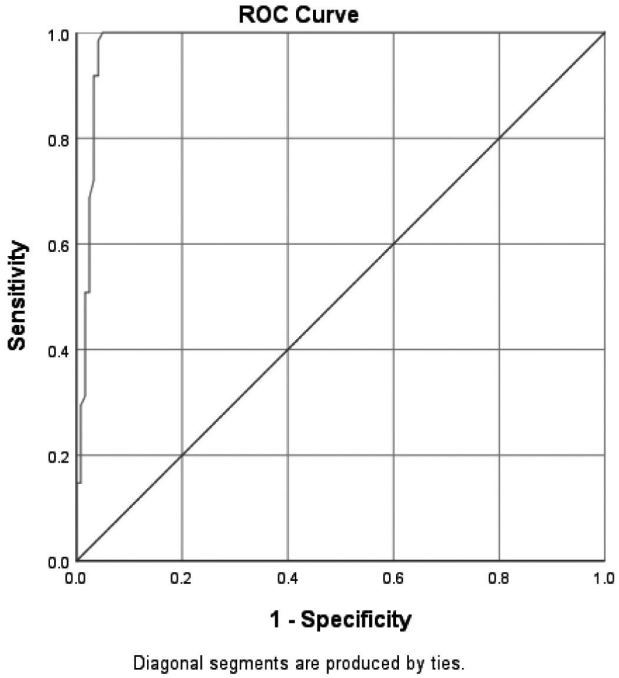

Figure 5. ROC Curve for Vaginal Fluid Creatinine

Table 8. Area under the curve for vaginal fluid creatinine

Area Under the Curve				
Test Result Variable(s): VagCrea				
Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.980	.010	.000	.960	1.000
The test result variable(s): VagCrea has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Using coordinates of the curve, the optimal cut off score that balances sensitivity with specificity was determined. The cut off score of 1.0050 was selected, with a 98.4% sensitivity/true positive rate, and 4.1% false positive rate.

Table 9 shows the accuracy values for the litmus paper, ferning, and vaginal fluid creatinine tests. Vaginal fluid creatinine with the cut off value set at 1.005 mg/dL had the highest sensitivity, specificity, positive likelihood ratio, positive and negative predictive values; and the lowest negative likelihood ratio of the 3 tests. The ferning and litmus paper tests only garnered 65.6% and 77% in terms of sensitivity, respectively, while vaginal fluid creatinine has 100% sensitivity.

The Pearson Correlation Coefficient test was performed on those with confirmed ruptured membranes to determine if gestational age affects the quantity of creatinine in vaginal fluid. The value obtained was 0.115, at a significance level of 0.378, indicating that increasing age of gestation only has a small positive correlation with the amount of vaginal fluid creatinine, that is not statistically significant (p = 0.378)

The Pearson correlation coefficient was also performed to determine the effect of time delay between physical exam and ruptured membranes (latency). The value obtained is -0.842 indicating that there is a negative correlation between vaginal fluid creatinine and hours of latency to physical exam, such that increasing time may lead to decreasing amounts of creatinine found in vaginal fluid, and this is statistically significant (p = 0.000) Figures 6 and 7 further demonstrate these correlations.

Table 9. Accuracies of the Litmus paper, Ferning, Vaginal fluid creatinine tests

Test involved	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Litmus	77%	90.2%	79.7%	88.7%	7.857	0.254
Ferning	65.6%	89.3%	75.5%	83.8%	6.131	0.385
Creatinine at 1.005 mg/dL	100%	95.1%	91%	100%	20.408	0

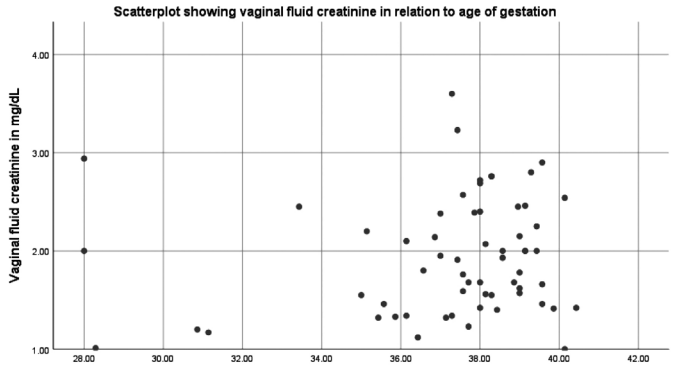

Figure 6

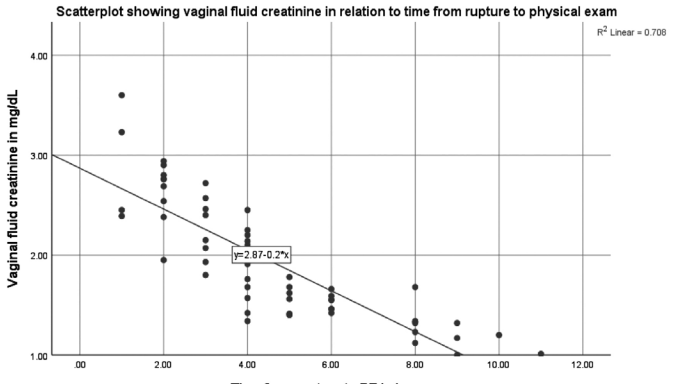

Figure 7

DISCUSSION

Upon evaluation, vaginal fluid creatinine set at 1.00 mg/dL has higher sensitivity, specificity, positive and negative predictive values, and a higher positive likelihood ratio than the litmus paper or ferning tests. The litmus paper test in the study had a lower sensitivity (77% vs 90-97%) and higher specificity (90.2% vs 16-70%) than in the references (Table 10). Also, the ferning test performed had sensitivities and specificities within the refence values[26]. This confirms the low accuracy values of these traditional tests fueling the search for more accurate tests.

When compared with previous studies dealing with vaginal fluid creatinine to detect prelabor rupture of membranes, this study had comparable accuracy values (Table 11) and confirmed the high diagnostic efficacy of this test.

The present study has one of the highest number of subjects among the studies on vaginal fluid creatinine as a marker for rupture of membranes. It has a similar cut off value to the studies conducted by El-Sabee (0.9 mg/dL), Hanfy (1.05 mg/dL), and Li (0.95 mg/dL)[17,19,28]. Based on the literature search, the sensitivity of vaginal fluid creatinine ranges from 46.6-100%, with majority of studies garnering a sensitivity of 94-100%, including the present study with 100%. Specificity for VFC ranges from 85-100%, the current study is within range at 95.1. The positive and negative predictive values are also consistently high among the studies. This means that the vaginal fluid creatinine test correctly identifies presence of rupture of membranes in most cases and only few cases may be missed (Table 11).

Tigli garnered a sensitivity of 46.6%, the lowest of all previous studies. A positive IGFBP-1 was used as the gold standard in this case, whereas in all others, rupture of membranes was confirmed by visualization of egress, or

Table 10. Performance of noninvasive tests to diagnose rupture of the fetal membranes[26]

Test/ Reference	Commercial Name	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Nitrazine (pH)	-	Positive/ Negative	90-97	16-70	63-75	80-93
Ferning	-	Positive/ Negative	51-98	70-88	84-93	87-97
AFP	ROM Check	>30 µg/L	90-94	95-100	94-100	91-94
Fetal fibronectin	Quick Check fFn	>50 ng/mL	97-98	70-97	74-93	98-100
IGFBP-1	ActimPROM, PROM-TEST, AMNI Check	>3 µg/L	74-97	74-98	73-97	56-95
Prolactin	-	>30-50 µIU/mL	70-95	76-78	72-84	75-93
Diamine oxidase	-	>25 µIU/test	83	90-100	100	89
B-HCG	-	40-65 µIU/mL	68-95	70-95	73-91	78-97
AmnioSense Absorbent pad		pH >5.2	98.3	70	65-70	98
Lactate	Lac Test	≥ 4.5 mmol/L	79-86	88-92	88-92	78-87
PAMG-1	AmniSure ROM Test	>5.0ng/mL	98-99	88-100	98-100	91-99

Table 11. Comparison of accuracy values of vaginal fluid creatinine from previous studies

Main Author	Proteins studied	Subjects	Cut-off (mg/dL)*	Sensitivity (%)*	Specificity (%)*	PPV (%)*	NPV (%)*	(+)LR (%)*	(-)LR (%)*
Current study Cortez 2017	Creatinine	183	1.00	100	95.1	91	100	20.4	0
Ghasemi 2016 [27]	Creatinine, Urea, Prolactin& beta-hCG	160	0.25	74.6	85	83	77.2	4.97	0.30
El-Sabee 2015[28]	Creatinine and Urea	96	0.9	100	85.9	78	100	7.11	0
Tigli 2014[16]	Creatinine, Urea, and beta-hCG	150	0.3	46.66	94.66	89.74	63.96	Not disclosed	Not disclosed
Kariman 2013 [20]	Creatinine and Urea	179	0.45	100	100	100	100	Not disclosed	Not disclosed
Zanjani 2012 [13]	Creatinine	180	0.5	96.7	100	100	96.8	Not disclosed	Not disclosed
Sekhavat 2012 [21]	Creatinine	160	0.14	98.7	100	100	98.8	Not disclosed	Not disclosed
Hanfy 2010 [17]	Creatinine, Urea	150	1.05	94	100	94	100	Not disclosed	Not disclosed
Kafali 2007 [15]	Creatinine and Urea	139	0.6	100	100	100	100	Not disclosed	Not disclosed
Gurbuz 2004 [14]	Creatinine	88	0.12	100	100	100	100	Not disclosed	Not disclosed
Li 2000 [19]	Creatinine, beta-hCG, AFP	88	0.95	90	100	100	90.9	Not disclosed	Not disclosed

pooling of amniotic fluid on speculum exam[16].

Other studies compared creatinine with other substances found in amniotic fluid. Tigli preferred β-HCG[16] and Ghesemi found prolactin and β-hCG have more diagnostic value than urea and creatinine in detecting PROM[27]. However, this is refuted by the study conducted by Li where creatinine was found cheaper and more accurate than β-hCG and AFP. It was further found that there is an overlap between values of AFP, hCG, and Fibronectin in patients with and without intact membranes making it a less useful test[19]. Kariman further confirmed that between urea and creatinine, creatinine assay has higher sensitivity and specificity, making it a better test[20]. Furthermore, the accuracy values of creatinine are comparable to more sophisticated and expensive tests like IGFBP-1 and PAMG-1[28].

Hence, most of the studies saw the superiority of vaginal fluid creatinine, considering it a reliable, simple, cheap, rapid test, excellent for screening, even considering it a possible gold standard for the detection of PROM.

Sekhavat included interval between sampling and delivery, and our study confirmed that with increasing time delay between rupture and physical exam there is less creatinine found in vaginal fluid. The longest time interval between exam and diagnosis is 12 hours beyond which creatinine was no longer detectable in amniotic fluid. There were no subjects included with ruptured membranes more than 12 hours.

Kafali speculated the use of creatinine for possible determination of fetal maturation, which was also demonstrated in our study, that with increasing age of gestation, there is increasing vaginal fluid creatinine, however this is not statistically significant.

CONCLUSION

Upon evaluation, vaginal fluid creatinine set at 1.00 mg/dL has higher sensitivity, specificity, positive and negative predictive values, and a higher positive likelihood ratio than the litmus paper or ferning tests. High accuracy values, with a low false negative rate of 0, and a large AUC make vaginal fluid creatinine an excellent test. This study confirms the findings from other studies that show the superiority of vaginal fluid creatinine, considering it a reliable, simple, cheap, rapid test, excellent for screening, even considering it a possible gold standard for the detection of PROM.

Additional findings showed that with increasing age of gestation, there is increasing vaginal fluid creatinine, however this is not statistically significant. Also that with increasing time delay between rupture and physical exam there is less creatinine found in vaginal fluid.

LIMITATION

This study is limited only to the study of creatinine in vaginal fluid of patients with singleton pregnancies within 28-40 weeks age of gestation, with no other comorbidities, with no contaminants in the amniotic fluid. It only compared the accuracy of vaginal fluid creatinine to traditional tests: ferning and litmus paper. Other studies included determination of Amniotic Fluid Index, but it was not done here.

RECOMMENDATION

The author recommends the use of vaginal fluid creatinine for the detection of prelabor rupture of membranes. Further studies should be conducted on the vaginal fluid creatinine of patients with multiple gestations, less than 28 and more than 40 weeks, with co-morbidities, and contaminants in amniotic fluid. Direct comparison with IGFBP-1 and PAMG-1 would also determine if it is a suitable cheaper alternative. The effect of increasing time interval beyond 12 hours on the amount of vaginal fluid creatinine should be investigated, as well as the effect of age of gestation on the amount of vaginal fluid creatinine.

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