Diagnosis of Premature Rupture of Membranes Using Vaginal Fluid Urea and Creatinine

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Research Article

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Abstract

Purpose: The diagnosis of premature rupture of membranes (PROM) can be difficult in equivocal cases. This study was designed to test the validity of vaginal fluid urea and creatinine in the diagnosis of PROM against the gold standard which is the Amnisure test.

Methods: The study was a prospective observational study. All consenting eligible pregnant women between gestational ages of 28 weeks to 41weeks + 6 days were recruited from the obstetrics emergency and antenatal clinic of the Federal medical centre, Keffi. Patients with a history of drainage of liquor were recruited as the case group, and controls that match for age, parity and gestational age were recruited from the antenatal clinic to constitute the control group. Vaginal fluid aspirated was assessed in the laboratory for urea and creatinine levels and an Amnisure test done. The sensitivity, specificity, negative and positive predictive values of vaginal fluid urea and creatinine were assessed in the diagnosis of PROM.

Results: Vaginal fluid urea and creatinine had sensitivity, specificity, Negative predictive value (NPV), Positive predictive value (PPV) respectively of 94%, 82%, 93.18%, 83.93% and 98%, 90%, 97.82%, 90.74%. The cut-off value for vaginal fluid urea and creatinine were 1.25 mg/dl and 20.3 mmol/l respectively.

Conclusions: This study has found that vaginal fluid urea and creatinine are very effective tests in diagnosis of PROM. It is a cheaper and more readily available alternative to the Amnisure test. It is especially useful in our environment, especially in cases of equivocal PROM, as a cost-effective means to confirm the diagnosis.

Introduction

Premature rupture of membranes (PROM) is defined as the rupture of the foetal amniotic membranes before the onset of labour [1–3]. It occurs in 10% of all term pregnancies, and is associated with a number of complications which include infection and preterm birth [1].

Rupture of foetal membranes occurs as a result of apoptosis, activation of some enzymes such as collagenase at 37weeks and mechanical forces [1]. PROM most likely occurs due to a premature initiation of these pathways [4]. Early PROM could also occur as a result of inflammation with or without infection of the membranes [4]. Studies have also suggested that membrane rupture could also be due to a number of factors such as defects in collagen structure and production as well as increased oxidative stress. Another factor implicated in PROM is a mismatch between production and breakdown of collagen in the extracellular matrix of the foetal membranes brought about by an increase in the activity of a number of matrix metalloproteins [1]. Several risk factors have been implicated in PROM and they include a low body mass index, urinary tract infections, a low socioeconomic status, vaginal bleeding and invasive procedures like amniocentesis [4, 5].

The correct diagnosis of PROM is essential. A wrong or missed diagnosis can lead to unnecessary interventions and delivery, as well as other adverse complications like infections to both mother and foetus [4, 6]. Routinely the diagnosis of PROM depends on a combination of an accurate history of a drainage or continuous trickle of fluid per vaginam. An examination findings of a pooling of clear fluid in the posterior fornix of the vagina, associated egress of fluid from the cervical canal, with a positive confirmatory test, for example Nitrazine test or Amnisure [7–10]. In indeterminate cases of PROM, these routine methods can lead to high false positive and false negative results. False positive Nitrazine test could be as a result of any lower genital tract infections or possible contamination of samples by semen, urine, and blood [7].

Many biochemical diagnostic modalities for PROM have been described. These include measurement of biomarkers with high concentrations in foetal amniotic fluid rather than other kinds of vaginal secretions, e.g. vaginal human chorionic gonadotropin (HCG), vaginal PH, vaginal fluid insulin growth factor binding protein-1, foetal fibronectin tests, vaginal fluid prolactin levels, urea, alpha fetoprotein (AFP) and creatinine [1, 11–16]. Even though these markers could improve the diagnosis of PROM, they have not become popular due to the complex nature of the tests and high cost [1].

Amnisure® is a newly developed easy to use, non-invasive diagnostic test of PROM and PPROM that combines both high sensitivity with low false positive results, it is designed as a dipstick format highly sensitive for detecting presence of placental alpha macroglogulin-1 (PAMG-1), a specific protein present in the amniotic fluid [17]. PAMG-1 was selected as a marker to use in Amnisure due to its unique characteristic presence in high levels in amniotic fluid, low level in blood, and extremely low in cervico-vaginal secretions, the test contains highly sensitive monoclonal antibodies that is designed to detect as low as 5ng/ml of PAMG-1 in cervico-vaginal secretion after a rupture of membranes [17].

There is a paucity of studies on the use of vaginal fluid urea and creatinine in the diagnosis of PROM in this environment. This study is apt because, measurement of urea and creatinine in vaginal washing for the detection of PROM is based on the concept that in the second half of a pregnancy, the major component of amniotic fluid is the urine of the foetus [1, 18]. Most of the current diagnostic tests for PROM have high sensitivity and specificity. Vaginal fluid urea and creatinine has values closest to Amnisure, clinical assessment, ferning test and Nitrazine test have a sensitivity of 85%, 97% and 93% respectively, and a specificity of 98%, 72%, and 85% respectively. Vaginal fluid urea and creatinine in diagnosis of PROM has a sensitivity of 98% and specificity of 100% [19].

In comparison to the Amnisure test, Urea and creatinine assay is readily available in most centres, is much cheaper to assay than the Amnisure test which costs $59.9 (#21000) per test kit [20], with no need for extra equipment and reagents, making introduction of this method into routine use feasible and practical. However, a major advantage the Amnisure test has over the urea and creatinine assay is that, the Amnisure test is a bedside test with results available in 10mins, while the urea and creatinine needs to be sent to the laboratory for assay and takes 1–2 hours to get the results. This study showed that vaginal fluid urea and creatinine was an effective and cheaper alternative to the Amnisure test.

Materials And Methods

This is a prospective observational study conducted with 50 pregnant women with PROM and 50 pregnant healthy controls who were seen in the department of obstetrics and gynaecology, Federal Medical Centre Keffi between September 2019 and March 2020. Pregnant women with gestational age between 28 + 0 and 41 + 6 were recruited for the study. Controls were recruited among women attending routine antenatal care. Women were excluded if they had vaginal spotting/bleeding, meconium in the vaginal fluid leak, having history of use of medications inserted into the vagina and sexual intercourse in the prior night.

Patients presenting to the obstetric emergency with a history of drainage of fluid per vaginam who met the inclusion criteria were recruited into the case group. For each patient recruited, a control patient was recruited from the antenatal clinic matching for age, parity and gestational age in consenting women, the first patient that matched was recruited. The demographic and obstetric features of women were recorded at the initial examination. Gestational age was calculated from the last menstrual period, in women who were unsure of date an early ultrasound scan was used to estimate gestational age.

Membrane rupture was diagnosed by the direct visualization of fluid leakage from the cervical canal or pooling of fluid in the posterior vaginal fornix. For the Amnisure test, the sterile polyethylene terephthalate swab supplied by the manufacturer was placed in the posterior fornix of the vagina. After a period of 1 min to ensure saturation, the swab was removed and agitated in the provided solvent vial for 1 min. The swab was then discarded and the Amnisure test strip placed in the vial. The sample in the vial was allowed to migrate through the test strip membranes by capillary action, and the test strip indicated a negative or positive result after a maximum of 10 min. The test strip was removed from the vial if two lines appeared (indicating a positive result) or after 10 min if only one line appeared (indicating a negative result). The manufacturer of the Amnisure test kit is the QIAGEN Company in the USA.

In the Patients with pooling of fluid in the posterior fornix, 3mls of the fluid was withdrawn with a sterile syringe. In the control group and in patients without obvious pooling in the posterior fornix, vaginal washing fluid for urea and creatinine sampling was done by instilling 5mls of sterile water for injection into the posterior vaginal fornix and 3 mls of it was withdrawn with the same syringe, the samples were then instilled into a lithium heparin bottle. Samples were taken to the laboratory for measurement, each sample at the lab was centrifuged at 50 revolutions/second and the supernatant fluid separated. All samples were collected by one of the researchers (AIA) within 6 hrs after membrane rupture before vaginal examination and administration of medications. Urea and creatinine levels were assessed by enzymatic urease method and Rate Jaffe method, respectively.

Data was entered into the statistical package for social sciences (SPSS) version 23 (IBM, Chicago, IL). Baseline initial frequency tables and charts were generated for univariate analysis. Analysis of numerical variables was performed by using independent student's t-test and categorical data by using Chi-squared test. Diagnostic accuracy was assessed using the following terms: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy. A receiver operating

characteristic (ROC) curve was drawn with the results of vaginal fluid urea and creatinine gotten, and the area under the curve (AUC) was used to estimate the sensitivity and specificity. A two by two table was drawn with the PROM positive and negative in the columns and vaginal fluid urea and creatinine positive and negative in the rows. This was used to calculate the PPV and NPV. The receiver operating characteristic curve was also used to establish an optimal cut-off concentration. The results were evaluated with a significance level of $p < 0.05$.

Results

This study consisted of 100 participants; 50 women with PROM and 50 women with healthy controls. Among the 50 total cases of PROM, 18 of them had no obvious pooling of fluid in the vagina.

As shown in Table 1, no significant differences were observed between the two groups in terms of mean age, gestational age and parity.

Table 2 shows vaginal fluid urea had a mean value of 2.6 ± 2.0 mg/dl in women with PROM as against 0.98 ± 0.35 mg/dl in the control group. Vaginal fluid Creatinine had a mean value of 97.55 ± 87.45 mmol/l in women with PROM as against 13.52 ± 5.55 mmol/l in the control group. Vaginal fluid urea and creatinine were both statistically significantly higher in women with PROM.

Table 3 shows vaginal fluid urea had a sensitivity, specificity, negative predictive value and positive predictive value of 94%, 82%, 93.18% and 83.93% respectively. Vaginal fluid creatinine had a sensitivity, specificity, negative predictive value and positive predictive value of 98%, 90%, 97.82% and 90.74% respectively. The Amnisure test had a sensitivity of 100% and specificity of 98%, with a NPV and PPV of 100% and 98.03% respectively. The Area under the curve (AUC) for vaginal fluid urea, vaginal fluid creatinine and the Amnisure test were 0.952, 0.979 and 0.99 respectively. Vaginal fluid urea and creatinine and the Amnisure test were all statistically significant in the diagnosis of PROM. Among the 50 total cases of PROM, 18 of them had no obvious pooling of fluid in the vagina.

Figure 1 shows the receiver operative characteristics curve for vaginal fluid urea and creatinine against the Amnisure test in the diagnosis of PROM. The X axis shows the false positive rate, while the Y axis shows the Sensitivity. Vaginal fluid Urea at a cut-off value of 1.25 mg/dl gives the highest sensitivity and specificity of 94% and 82% respectively. Vaginal fluid creatinine at a cut-off of 20.3 mmol/l had a sensitivity of 98% and specificity of 90%. The Amnisure test had a sensitivity of 100% and specificity of 98%.

Table 1
The demographic characteristics of groups

PARAMETERS	PROM + case group mean ± SD (Range)	PROM -control group mean ± SD (Range)	p VALUE
AGE (years)	28.32 ± 5.45 (16–38)	28.18 ± 5.31 (17–39)	0.307
PARITY	1.84 ± 1.69 (0–6)	1.86 ± 2.02 (0–9)	0.463
GESTATIONAL AGE (weeks + days)	34.9 ± 3.43 (28–41 + 2)	34.33 ± 3.6 (28 + 2–40 + 6)	0.328

Table 2
Vaginal fluid urea and creatinine level among groups

PARAMETERS	PROM + case group mean ± SD (Range)	PROM -control group	p VALUE
Vaginal fluid Urea {mg/dl)	2.6 ± 2.0 (0.6–16)	0.98 ± 0.35 (0.5–2.6)	< 0.001
Vaginal fluid Creatinine(mmol/l)	97.55 ± 87.45(9.4-627.7)	13.52 ± 5.55 (2.8–35.3)	< 0.001

Table 3
ROC curve analysis for vaginal fluid urea and creatinine in diagnosis of PROM against the Amnisure test.

PARAMETERS	ROC RESULTS				Cut off	AUC	p Value
	Sensitivity	Specificity	NPV	PPV			
Vaginal fluid Urea {mg/dl)	94%	82%	93.18%	83.93%	1.25	O.952	< 0.001
Vaginal fluid Creatinine(mmol/l)	98%	90%	97.82%	90.74%	20.30	0.979	< 0.001
Amnisure Test	100%	98%	100.%	98.03%		0.990	< 0.001

Discussion

The importance of a prompt and effective means of diagnosis of premature rupture of membranes cannot be overemphasized [1]. A delay in diagnosis can lead to a variety of maternal and foetal complications which include chorioamnionitis and neonatal sepsis [1].

This study found that the mean value for vaginal fluid urea was 2.6 ± 2.0 mg/dl in the women with PROM as against 0.98 ± 0.35 in the control group. Vaginal fluid creatinine had a mean value of 97.55 ± 87.45 mmol/l in women with PROM as against 13.52 ± 5.55 in the control group. The values were both statistically significant. These were similar to findings in studies by Begum et al, Zanjani et al and Kuruoglu et al who found a statistically significant difference in values of vaginal fluid urea and creatinine between women with PROM and the control group [1, 19, 21]. This could be as a result of

similar levels of urea and creatinine in urine of foetuses generally across populations, and similar levels in other body fluids.

This study found the cut-off value for vaginal fluid urea and creatinine in diagnosis of PROM to be 1.25 mg/dl and 20.3 mmol/l respectively. Begum et al found a similar cut-off value of 26.4 mmol/l for vaginal fluid creatinine but found a higher cut off of 6 mg/dl for vaginal fluid urea [1]. Zanjani et al found a cut off for vaginal fluid creatinine of 44 mmol/l [19], which was higher than the cut off value in this study. The differences in the cut off value could be as a result of different sample sizes, inclusion criteria and gestational ages of recruited participants between the studies.

This study found that both vaginal fluid urea and creatinine were effective in the diagnosis of PROM. At a cut-off value of 1.25 mg/dl, vaginal fluid urea had the highest values for both sensitivity and specificity. The sensitivity was 94% and specificity was 82%, with a NPV and PPV of 93.18% and 83.93% respectively. Begum et al, found slightly higher values for vaginal fluid urea with a sensitivity, specificity, NPV and PPV of 98%, 100%, 98.4%, and 99% respectively [1]. This could be due to slight variations in results as a result of different analysers being used in both studies. The differences could also be due to inter observer error in reading the result.

Vaginal fluid creatinine had the highest sensitivity and specificity at a cut-off value of 20.3 mmol/l. The sensitivity, specificity, NPV and PPV at this cut-off level were 98%, 90%, 97.82%, 90.74% respectively. This was similar to findings by Zanjani et al, who found vaginal fluid creatinine to have a sensitivity, specificity, NPV and PPV of 96.7%, 100%, 96.8%, 100% respectively [19]. Begum et al also had similar findings with a sensitivity, specificity, NPV and PPV of 90%, 93.83%, 90.74%, 97.83% respectively [1]. Kuruoglu et al had similar findings of 94.4%, 93.3%, 97.7% and 85% respectively [21].Altough the studies recruited women across different groups of gestational ages, they still had similar findings. These similarities could be as a result of a similar foetal urine content of creatinine. This finding is significant as it shows that vaginal fluid creatinine assay above 20.3 mmol/l could be used as a diagnostic marker for PROM especially in women with clinically equivocal PROM.

The Amnisure test had a sensitivity, specificity, NPV and PPV of 100%, 98%, 100%, 98.03% respectively. Eleje et al found a slightly lower sensitivity and specificity of 97.4% and 96.7% [22]. This may be as a result of the larger study population in the study by Eleje et al.

This study found that the area under the curve (AUC) for vaginal fluid urea and creatinine were 0.952 and 0.979 respectively and these were comparable to that of the Amnisure test which was 0.99. This showed that both vaginal fluid urea and creatinine were very good biomarkers in diagnosis of PROM. This study therefore shows that both vaginal fluid urea and creatinine can be used as an accurate diagnostic marker for PROM especially in women who have clinical equivocal cases of PROM. Begum et al reported similar findings with an AUC of 0.952 for vaginal fluid urea and 0.99 for creatinine [1].

Conclusion

PROM remains a leading cause of preterm births and its antecedent problems. The correct diagnosis of PROM is critical for both maternal and foetal concerns. While a false positive diagnosis leads to unnecessary hospitalization, a false negative diagnosis causes intrauterine infection, increasing morbidity and mortality of both mother and foetus.

Vaginal fluid urea at a cut of value of 1.25 mg/dl and Creatinine at a cut of value of 20.3 mmol/L are accurate, cheap and simple methods in diagnosing PROM as their Sensitivity, specificity, PPV, NPV were 94%, 82%, 83.93%, 93.18% and 98%, 90%, 90.74%, 97.82% respectively.

Currently the Amnisure test is the most valuable and effective means of diagnosis of PROM. This study has found that vaginal fluid urea and creatinine are very effective tests in diagnosis of PROM, with accuracy similar to that of the Amnisure test. It is a cheaper and more readily available alternative to the Amnisure test.

Limitations Of The Study

Limitations of the study included recall bias especially with history of exclusion criteria such as sexual intercourse in the prior night, previous history of vaginal infections or vaginal bleeding. There was also difficulty in dating of pregnancy in women who were unsure of date.

Declarations

Author contributions

AIA: Conceptualization, original manuscript writing, data collection, data analysis/interpretation, and funding. AFA: Conceptualization, supervision, manuscript review and editing. UAA: Conceptualization, supervision, manuscript review and editing. OAT: Supervision, manuscript review and editing. GTI: Manuscript review and editing. OSO: Manuscript editing, proof reading.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest

Ethical approval Ethical approvals were obtained from the Research and Ethical Committee of the Federal Medical Centre Keffi, Keffi, Nasarawa State.

Informed consent Written informed consent was obtained from the patients for publication.

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Figures

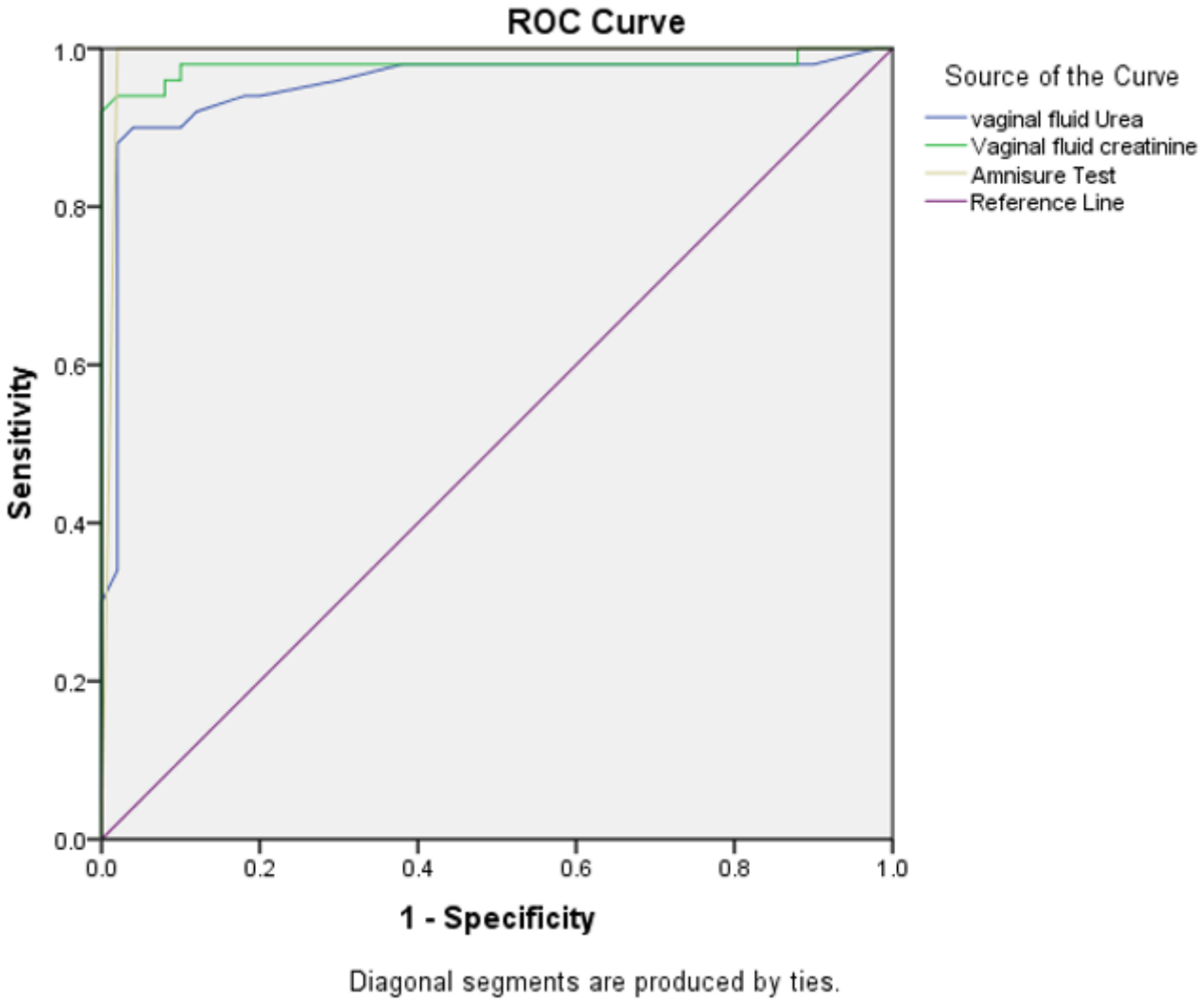

Figure 1

ROC Curve for Vaginal fluid Urea and creatinine against Amnisure test in Diagnosis of PROM