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Original Article

Comparative diagnosis of premature rupture of membrane by nitrazine test, urea, and creatinine estimation

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ABSTRACT

Objective: The study compared the reliability of nitrazine, urea, and creatinine in the diagnosis of premature rupture of membrane (PROM). Nitrazine strip measures pH levels while urea and creatinine are produced mainly in amniotic fluid and not in the maternal vagina.

Methods: Sixty-four pregnant women with demonstrable passage or pooling of liquor par vaginum on speculum examination and 64 with no liquor drainage at 28–42 weeks gestation were studied. Vaginal fluid aspirates from both groups of patient were tested for urea, creatinine, and pH levels using nitrazine strip. Receiver Operating Characteristic (ROC) curves were plotted to determine cutoff values for urea and creatinine. Statistical analysis was done using SPSS version 23.0 and setting statistical significance at $P \leq 0.05$.

Results: Nitrazine test showed high level of specificity (100%) but a sensitivity of 87.5%. Predictive ability of urea at >12.7 mg/dl obtained as cutoff value from the ROC curve showed a sensitivity of 19.64% and specificity of 94.44% while for creatinine the cutoff value was at >0.8 mg/dl with sensitivity of 48.21% and specificity of 65.28%.

Conclusion: Nitrazine strip was the most reliable of the three in the diagnosis of PROM. This was followed by creatinine and then urea. In view of the gaps in sensitivity and accuracy and the importance of precise diagnosis to prevent maternal and fetal complications, there is still the need to find other affordable, more sensitive and more accurate biochemical marker/s that will help in diagnosing PROM especially in difficult cases.

Keywords: Creatinine, nitrazine specificity, sensitivity, urea

Introduction

Premature rupture of membrane (PROM) can be defined as rupture of membranes before onset of labor, if this occurs before 37 weeks of gestation it is defined as preterm PROM (PPROM) and if at 37 weeks and beyond as PROM or term PROM.[1,2]

The incidence of PROM said to be between 5% and 10% of all deliveries while that of PPROM is put at 3% and it is the cause of over a third of preterm deliveries.[3,4] It is a significant cause of maternal and perinatal morbidity and mortality. Risk factors include polyhydramnios, low socioeconomic status, and low body mass index. There was no increased risk of PROM among obese patients in Ahmed *et al*.'s study.[5] Maternal effects include endometritis, salpingitis, and sepsis.[6] Fetal complications may include fetal lung hypoplasia, umbilical cord compression, chorioamnionitis, neonatal sepsis, and many others.[7,8]

Patients history may suggest PROM, although this has been shown to be reliable only in 10–50% of patients.[9,10] This maybe because the significance or perception of the illness by the women may be inadequate.[11] Observation of fluid leakage from cervix or accumulation in posterior fornix on speculum examination has been the main method for definite diagnosis of PROM. Other diagnostic measures used include pH test and microscopic examination of amniotic fluid. Amniotic fluid typically has a pH of 7.1–7.3, while normal vaginal secretions have a pH of 4.5–6.0. pH test can be done by use of nitrazine strips which turns dark blue from yellow in fluids with pH above 6.5.[12] False-positive nitrazine may occur in presence of blood, semen, infections such as bacterial vaginosis. Diagnostic challenge may arise in absence of demonstrable egress or accumulation of fluid on speculum examination. Other confirmatory tests include ultrasound guided Instillation of indigo carmine dye into the uterus and observation of a blue stain on perineal pad or tampon.[2] This is however invasive and is associated with risks of intrauterine infection, bleeding, iatrogenic PROM and miscarriage.

Recent studies have focused on biochemical agents with high concentration in amniotic fluid but little or no quantity

in cervicovaginal secretions.[13-16] Examples include insulin like growth factor binding protein-1, fetal fibronectin Lactate and beta-subunit of human gonadotropin and placental alpha macroglobulin. Studies on use of metabolomics to predict preterm births are also on going.[17] However, cost and availability of required tests for these substances are important challenges in our local settings and areas where tertiary care is not always affordable or available.[18] Possibility of using urea and creatinine in the diagnosis of PROM was explored because of the relative ease and affordability of testing of these electrolytes. Moreover, it is expected that accuracy of these test should be higher. Amniotic fluid creatinine increases from 20 to 32 weeks and urea in third trimester while they are absent in cervicovaginal fluid.[16] The aim of this study was to compare reliability of nitrazine paper, urea, and creatinine in the confirmation of PROM.

Methods

Study background

This study was done over a period of 7 months in the Obstetrics and Gynaecology unit of University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria, while the chemical analysis was carried out in the chemical pathology department. Ethical approval was obtained from the University of Ilorin Teaching hospital ethical review committee (Approval number: NHREC/02/05/2010). Written informed consent was also obtained from all participants.

Study design

This was a comparative study.

Study population

The study population consisted of pregnant women between 28 and 42 weeks gestational ages. Study group was those who presented with history and demonstrable passage of liquor per vaginum and speculum examination revealed pooling of fluid in the posterior fornix of the vagina or trickling of fluid from the cervix with or without valsalva maneuver but with no uterine contractions. The control group was gestational age matched pregnant women without history or clinical evidence of drainage of liquor.

Inclusion criteria

Pregnant women with gestational age 28–42 weeks with confirmed demonstrable PROM and controls with no history nor suspicion of PROM.

Exclusion criteria

History suggestive of vaginitis, history or presentation with antepartum hemorrhage, Urinary incontinence, polyhydramnios, chorioamnionitis, or coitus within 24 h before presentation were excluded from the study. Others include history of intake of antibiotics or use of local pessaries as well as recent vaginal examination or use of vaginal douches.

Sample size determination

The sample size was calculated using the Fischer formula.[19]

$$n = z^2pq/d^2$$

Where:
n=minimum sample size for the study
z= standard normal deviation usually set at 1.96 at 95% confidence interval.
p= the prevalence of PROM. For this study, a prevalence of 3.9%[20] was used.
q=1-p=1-0.039=0.961.
d= the degree of accuracy desired, usually set at 0.05.
$n=(1.96)^2 \times 0.039 \times 0.961/(0.05)^2$
n=57.59
n=58 was the minimum sample size.

To cater for attrition, 10% of the sample size was added to the initial sample size which was 6. Hence, sample size was 64 in each arm of the study; 64 in the PROM group (Group I), and 64 in the control group (Group II). This gave a total sample size of 128.

Sampling method

Purposive non-probability sampling was employed.

Study procedure

Patients were recruited from the antenatal, emergency, labor wards, and antenatal clinics of the department. A history of the index pregnancy was obtained including booking status and antenatal care received so far. The records of booked patients were reviewed. Participants and controls underwent a general physical examination as well as sterile speculum examination and Nitrazine testing. A positive Nitrazine test was a change in the color of nitrazine paper from yellow to blue. All these were recorded in the pro forma which was the research instrument.

Sterile vaginal speculum examination, nitrazine testing, and sample collection

After detailed explanation of the study and procedure informed consent was obtained. Sterile vaginal speculum examination was done for every patient.

In the study group, PROM was confirmed by visualizing pooling of fluid in the posterior fornix or trickling of fluid from the cervical os on Valsalva maneuver. The liquor was checked for color and smell, 3 ml was aspirated and put inside a sterile bottle for laboratory analysis. A cotton tip applicator was then dipped inside the pool of liquor and applied to a nitrazine paper for testing.

In controls, speculum examination was done as described above, the posterior fornix was irrigated with 5 mls of sterile water and 3 ml was aspirated, put in a sterile bottle and sent for laboratory analysis. A cotton tipped swab was also applied to the remaining sterile water in the posterior fornix and put on a nitrazine paper for testing. Samples were taken to the chemical pathology laboratory for analysis of urea and creatinine levels.

Urea and creatinine estimation

Urea estimation was based on the principle of enzymatic degradation by urease while creatinine was based on reaction with picric acid. These reactions produced color changes, which were measured by absorbance spectrophotometer against standard solutions of known concentration of urea and creatinine.

Data management

Analysis was performed using SPPSS version 20.0 and $P < 0.05$ was termed significant. The data were presented in frequency tables, histogram, and curve graphs. Chi-square analysis and odds ratios with 95% confidence intervals were used to compare proportions and Student's t-test for continuous data.

Results

The socio-demographic and obstetric variables of the participants are shown in Table 1. The age range of participants was 20–44 years. The mean age of the subjects was 30.55 ± 4.92 years while it was 31.03 ± 5.38 years for the controls.

The majority of the subjects were self-employed while most of the controls were organized public or private sector employees. The self-employed comprised of the artisans, traders and business women. Of the subjects 27 (42.2%) were either public or private sector employees (employed) while only 15 (23.4%) were unemployed. On the other hand, 33 controls (51.6%) were either public or private sector employees while only 9 controls (14.1%) were unemployed.

A total of 104 participants (81.3%) were booked, of these 43 (67.2%) were subjects while 61 (95.3%) controls were booked. The booking status of all the participants was statistically significant, $P < 0.001$.

Duration of liquor drainage before presentation is shown in Figure 1. Majority 42 (65.6%) of the subjects presented within 24 h of PROM, while 5 (7.8%) presented after 72 h of PROM, the least number 4 (6.3%) presented between 48 and 72 h after onset of drainage of liquor.

Participants that presented between 48 and 72 h after onset of drainage of liquor had the highest median level of vaginal fluid urea of 5.85 mg/dl (IQR 2.18–8.40 mg/dl) while those presenting between 24 and 48 h had the least median level of 3.90 mg/dl (IQR 0.8–13.2 mg/dl). The median vaginal fluid creatinine level was highest in those presenting between 24 and 48 h, 1.1 mg/dl (IQR: 0.3–2.25 mg/dl) while it was least in those who presented after more than 72 h, 0.3 mg/dl (IQR 0.1–2.0 mg/dl). The duration of PROM had no significant effect on the vaginal fluid level of urea ($P = 0.829$) or creatinine ($P = 0.634$), this is shown in Table 2.

Table 3 reveals that the percentage of positive Nitrazine test was highest within 24 h of PROM, it then reduced gradually with increasing duration of PROM, while the likelihood of getting a false negative test in the presence of PROM increased with duration of liquor drainage from 9.5% at >24 h to 25% at 48–72 h PROM. Surprisingly all five cases with PROM duration >72 h were all truly positive. These changes were however not significant. Neither urea or creatinine levels nor nitrazine test were significantly affected by duration of PROM.

Cutoff values for urea and creatinine in predicting PROM for this study was determined using the Receiver Operating Characteristic Curve (ROC), this is shown in Figure 2. The area under the curve (AUC) of the ROC curve for urea was 0.503 at $P = 0.957$. The AUC helps to determine the discriminative power of a test and is a measure of diagnostic accuracy. The

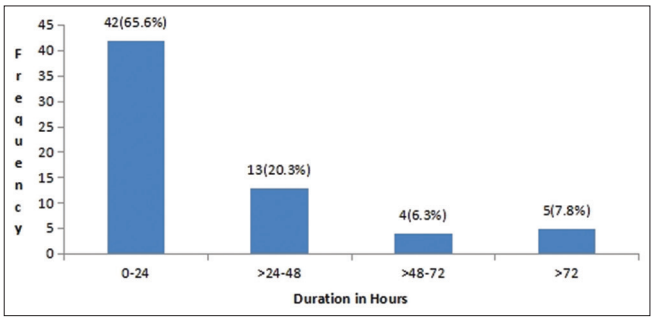

Figure 1: Duration of drainage of liquor to presentation in hospital

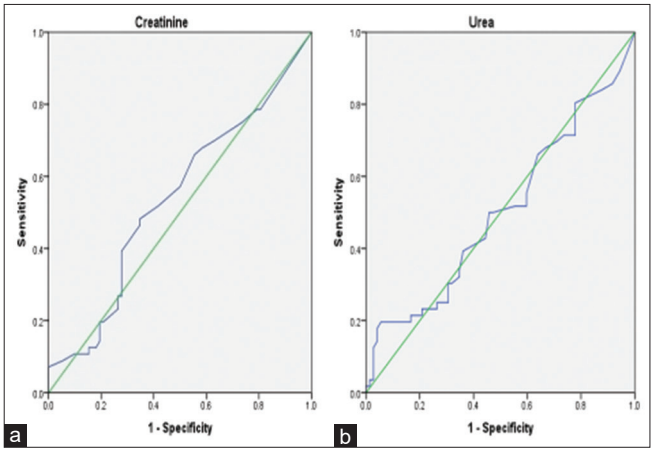

Figure 2: (a and b) Receiver operating characteristic curves for urea and creatinine. Area under the curve: 0.503; *p* value: Area under the curve: 0.537; *p* value: 0.475; 0.957; 95%Confidence interval:0.413–0.592; 95%Confidence interval: 0.447–0.626

Table 1: Socio demographic and obstetric variables of the participant

Variables	Subject *n* (%)	Control *n* (%)	Total *n* (%)	χ^2/t	*P*-value
Age group (years)					
<25	9 (14.1)	8 (12.5)	17 (13.3)	1.586	0.811
26–30	27 (42.2)	25 (39.1)	52 (40.6)		
31–35	17 (26.6)	16 (25.0)	33 (25.8)		
36–40	9 (14.1)	14 (21.9)	23 (18.0)		
>40	2 (3.1)	1 (1.6)	3 (2.3)		
Mean±SD	30.55±4.92	31.03±5.378		0.532[t]	0.596
Educational status					
None	4 (6.3)	1 (1.6)	5 (3.9)	2.993[Y]	0.393
Primary	1 (1.6)	5 (7.8)	6 (4.7)		
Secondary	16 (25.0)	11 (17.2)	27 (21.1)		
Tertiary	43 (67.2)	47 (73.4)	90 (70.3)		
Ethnicity					
Yoruba	59 (92.2)	56 (87.5)	115 (89.5)	1.660[Y]	0.646
Hausa/Fulani	2 (3.1)	0 (0.0)	2 (1.6)		
Igbo	1 (1.6)	2 (3.1)	3 (2.3)		
Others	2 (3.1)	6 (9.4)	8 (6.3)		
Occupation					
Employed	22 (34.4)	33 (51.6)	55 (43.0)	4.210	0.122
Self employed	27 (42.2)	22 (34.4)	49 (38.3)		
Unemployed	15 (23.4)	9 (14.1)	24 (18.8)		
Gestational age (weeks)					
28–32	11 (17.2)	17 (26.6)	28 (21.9)	2.484	0.289
33–36	20 (31.3)	22 (34.4)	42 (32.8)		
37–42	33 (51.6)	25 (39.1)	58 (45.3)		
Mean±SD	35.84±3.58	34.75±3.55		1.737t	0.085
Gravidity					
1	21 (32.8)	12 (18.8)	33 (25.8)	3.664	0.160
2–4	29 (45.3)	38 (59.4)	67 (52.3)		
≥5	14 (21.9)	14 (21.9)	28 (21.9)		
Booking status					
Booked	43 (67.2)	61 (95.3)	104 (81.3)	16.615	<0.001*
Unbooked	21 (32.8)	3 (4.7)	24 (18.8)		

χ^2: Chi square; Y: Yates corrected Chi-square; t: Independent Samples *t*-test

cutoff value of urea for predicting PROM as determined from the ROC was at >12.7 mg/dl with sensitivity of 19.64% and specificity of 94.44%.

The AUC of the ROC for creatinine was 0.537 at $P = 0.475$. The cutoff value for predicting PROM as determined from the ROC curve was at >0.8 mg/dl with sensitivity of 48.21% and specificity of 65.28%.

Table 4 shows the percentage of positivity and negativity obtained using nitrazine, urea, and creatinine and the measure of agreement tests. Nitrazine was positive in 56 out of 64 cases in the study group and negative in the entire control group. This test result was statistically significant with $P = 0.04$.

At the determined cut of value of 12.7 mg/dl, urea was positive in only 12 cases of study group and negative in 52 cases. Among the controls 61 (95.3%) were truly negative for urea. Creatinine was positive in 31 (48.4%) of the 64 study group participants and falsely negative in 33 (51.6%) while of the control group, it was truly negative in 43 (67.2%) and falsely positive in 21 (32.8%). The k value of 0.875 of nitrazine showed that it was the most reliable test for predicting PROM, followed by creatinine 0.156 and finally urea at k of 0.141.

Table 5 shows the value of the various tests in detecting and eliminating PROM. Specificity is defined as the ability of a test to correctly identify people without the disease, while

Table 2: Comparing vaginal fluid urea and creatinine levels with duration of PROM among study group participants

Variable	Urea median (IQR)	Creatinine median (IQR)
Duration of PROM (h)		
0–24	5.40 (2.25–10.38)	0.80 (0.2–2.00)
>24–48	3.90 (0.80–13.20)	1.10 (0.30–2.25)
>48–72	5.85 (2.18–8.40)	0.60 (0.53–0.68)
>72	5.50 (0.90–6.90)	0.30 (0.10–2.00)
K/F (*P*-value)	0.885[K] (0.829)	1.712[K] (0.634)

IQR: Inter-quartile range K: Kruskal–Wallis test

Table 3: Nitrazine positivity with duration of PROM among study group participants

Duration of drainage (h)	Nitrazine test			χ^2	*P* value
	Positive	Negative	Total		
	n (%)	*n* (%)	*n* (64)		
0–24	38 (90.5)	4 (9.5)	42	2.956	0.399
>24–48	10 (76.9)	3 (23.1)	13		
>48–72	3 (75.0)	1 (25.0)	4		
>72	5 (100.0)	0 (0.0)	5		
Median (IQR)	12.50 (6.25–45.50)	33.50 (13.50–48.00)			
Range	2–120	12–72		134.500[U]	0.069

χ^2: Chi square test; U: Mann-Whitney U test. IQR: Inter-quartile range

Table 4: Nitrazine test versus vaginal fluid urea and creatinine levels of study participants in evaluating PROM

Variable	Study group	Control	K	*P*	OR (95% CI)
	n=64 (%)	*n*=64 (%)			
Nitrazine test					
Positive	56 (87.5)	0 (0.0)	0.875	0.042	9.000 (4.683–17.297)
Negative	8 (12.5)	64 (100.0)			
Urea (>12.7 mg/dl)					
Positive	12 (18.8)	3 (4.7)	0.141	0.056	4.692 (1.256–17.532)
Negative	52 (81.3)	61 (95.3)			
Creatinine (>0.8 mg/dl)					
Positive	31 (48.4)	21 (32.8)	0.156	0.086	1.924 (0.940–3.936)
Negative	33 (51.6)	43 (67.2)			

K: Measure of agreement (Kappa); OR: Odds Ratio; CI: 95% Confidence interval

the sensitivity of the test is the ability of the test to correctly identify people with the disease. Nitrazine test showed high level of specificity (100%) but a sensitivity of 87.5%. The next more sensitive test was creatinine test while urea had the lowest sensitivity of the 3.

Table 5: Evaluation of nitrazine and the cutoff points of urea and creatinine in the diagnosis of PROM

Evaluation	Nitrazine	Urea test (>12.7 mg/dl)	Creatinine (>0.8 mg/dl)
Sensitivity	87.5%	18.8%	48.4%
Specificity	100%	95.3%	67.2%
Positive Predictive Value	100%	80.0%	59.6%
Negative Predictive Value	88.9%	54.0%	56.6%
False Positive rate	0%	4.7%	32.8%
False Negative rate	12.5%	81.3%	51.6%
Accuracy	93.75%	57.0%	57.8%

Discussion

Prompt and accurate diagnosis of PROM preterm and at term is important, although management modalities vary. While conservative approach of management may be employed for preterm, for term PROM (37–42 weeks) stimulation of labor with oxytocin or prostaglandin may be necessary if spontaneous onset of labor fails to occurs, to avoid prolonged PROM, chorioamnionitis and other complications. There may be associated antimicrobial use or misuse.[21,22]

A larger percentage (65%), of PROM cases presented before 24 h of drainage while the remaining 35% presented after 24 h of drainage. This is similar to what was obtained by Sharma *et al*. and Khan *et al*. in which 91.6% and 61.67%, respectively, presented before 24 h of drainage.[6,23]

Vaginal fluid levels of urea and creatinine showed no significant difference with duration of PROM, with $P = 0.829$ and 0.634 respectively, this may be due to the relative stability of urea and creatinine in the vaginal fluid as compared to the proteins in ferning test and fetal cells which may be readily degraded by microorganisms in the vagina flora which are known to produce proteases.[24] With nitrazine, there was a definite, although also not statistically significant difference of increased positivity with shorter duration of PROM. Percentage of false negative increased with longer durations of PROM. However, in the five cases longer than 72 h, nitrazine test was surprisingly positive in all, these cases could possibly be those with intermittent leakage of liquor.

The optimal cutoff value for urea for this study was 12.7 mg/dl. This cutoff value is comparable to 13.2 mg/dl obtained by Mohammed *et al*.[10] and 14.75 mg/dl reported by Hanfy.[25] However, it is higher than the 6.0 mg/dl reported by Kariman *et al*.[26] The AUC obtained from the ROC curve

for urea was 50.3% (0.503). This finding is much lower when compared to the AUCs of 84% and 91% reported by Kariman *et al*.[26] and Hanfy,[25] respectively. The values for sensitivity, specificity, PPV, NPV, and accuracy of vaginal fluid urea level from this study was 18.8%, 95.3%, 80.0%, 54.0%, and 57.0%, respectively, while other studies were 90%, 79%, 83%, 87.5%, and 85%, respectively, by Kariman *et al*.,[26] 96% and 93%, respectively, for sensitivity and specificity of urea and 97% and 98%, respectively, for creatinine by Malchi *et al*.,[27] 100% for all the parameters by Hanfy[25] and Mohammed *et al*.[10]

The optimal cutoff for creatinine level in vaginal fluid was 0.8 mg/dl. This figure is lower than 1.05 mg/dl obtained by Hanfy[25] but it is higher than 0.05–0.5 mg/dl reported by others.[26,28,29] The AUC from the ROC curve for creatinine from this study was 53.7% (0.537). This is in contrast to the AUCs of 99.99% by Kariman *et al*.[26] and 82% by Hanfy.[25] For creatinine, sensitivity, specificity, PPV, NPV, and accuracy obtained from the study are 48.2%, 65.3%, 51.9%, 61.8%, and 57.8%, respectively. The values reported for sensitivity, specificity, PPV, NPV, and accuracy for creatinine in other studies ranged between 90 and 100%[10,19-22] Several studies obtained 90% and above for sensitivity, specificity PPV and NPV of urea and creatinine.

Sensitivity, specificity, PPV, NPV, and accuracy of nitrazine test for PROM in this study were 87.5%, 100%, 100%, 88.9%, and 93.7%, respectively. This is similar but slightly higher than in the study by Agbara *et al*. in which nitrazine had an accuracy of 89.3%.[30] It was also comparable to the study by Haseli *et al*. with the values 94.1%, 90.5%, 98.2%, and 73.1%.[31]

Urea and creatinine have not been shown to be reliable in predicting PROM this is because the AUC obtained from the ROC curves are low 50.3% (0.503) for urea and 53.7% (0.537) for creatinine suggest unreliability. The sensitivity levels (the ability of the test to correctly identify the disease) are also low, urea's sensitivity was 18.8%, and creatinine sensitivity was 48.3%.

These results are quite different from several other studies as mentioned above. Possible reasons for such low values could be a result of interference from components of amniotic fluid[32,33] the influence of these interfering substances could be complex interfering either negatively or positively. Furthermore, ethnicity, differences in selection criteria and laboratory techniques could also possibly be factors responsible for the differences. Of the two, creatinine showed to be more reliable than urea in predicting PROM (κ values of 0.156 and 0.141 and diagnostic accuracy of 57.8% and 57.0% respectively). This is similar to the finding of Kariman *et al*.[26] However Hanfy reported that[25] urea was more reliable than creatinine while Mohammed *et al*.[2] discovered that they were both of equal reliability.

Nitrazine, on the other hand, remains a reliable instrument for detection of PROM and especially with proper selection of patient. In the analysis of the relationship between the three methods, Nitrazine had a k value of 0.875 which is fairly close to 1 and much higher than was obtained for urea and creatinine.

Conclusion

According to our findings, nitrazine was more reliable than vaginal fluid levels of urea and creatinine in the diagnosis of PROM, but, creatinine is comparatively more reliable than urea in the diagnosis of PROM. Further research efforts should be geared towards perfecting urea and creatinine estimation with minimal interference and finding other new novel but affordable biochemical markers with higher sensitivity in the vaginal fluid that will help in diagnosing PROM in difficult cases.

Authors' Declaration Statements

Ethics approval and consent to participate

Ethical approval was obtained from the University of Ilorin Teaching hospital ethical review committee (Approval number: NHREC/02/05/2010). Written informed consent was also obtained from all participants.

Consent for publication

None.

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

Study conception and design and data collection: Dr. Olaomo, Prof Adesina; Analysis and interpretation of results: Dr. Olaomo and Olarinoye A.O; manuscript preparation and revision: Dr. Olarinoye, Ezeoke, Prof Aboyeji; all authors reviewed the results and approved the final version of the manuscript.

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References

1. Dayal S, Hong PL. Premature Rupture of Membranes. Treasure Island, FL: StatPearls Publishing; 2020.
2. Caughey AB, Robison JN, Norwitz ER. Contemporary diagnosis and management of preterm premature rupture of membrane. Rev Obstet Gynecol 2008;1:11-22.
3. Assefa NE, Berhe H, Girma F, Berhe K, Berhe YZ, Gebreheat G, *et al*. Risk factors of premature rupture of membranes in public hospitals at Mekele city, Tigray, a case control study. BMC Pregnancy Childbirth 2018;18:386.
4. Idrisa A, Pius S, Bukar M. Maternal and neonatal outcomes in premature rupture of membranes at University of Maiduguri Teaching Hospital, Maiduguri, North-Eastern Nigeria. Trop J Obstet Gynaecol 2019;36:15-20.
5. Ahmed SR, Ellah MA, Mohamed OA, Eid HM. Prepregnancy obesity and pregnancy outcome. Int J Health Sci (Qassim) 2009;3:203-8.
6. Sharma SK, Dey M. Maternal and neonatal outcome in cases of premature rupture of membranes beyond 34 weeks of gestation. Int J Reprod Contracept Obstet Gynecol 2017;6:1302-5.
7. Ismail AQ, Lahiri S. Management of prelabour rupture of membranes (PROM) at term. J Perinat Med 2013;41:647-9.
8. ACOG Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin No. 80: Premature rupture of membranes. Clinical management guidelines for obstetrician-gynecologists. Obstet Gynecol 2007;109:1007-19.
9. Wilberg-Itzel E, Cnattingius S, Nordstrom L. Lactate determination in vaginal fluids: A new method in the diagnosis of prelabour rupture of membranes. BJOG 2005;112:754-8.
10. Mohamed A, Mostafa W. The value of measurement of vaginal fluid urea, creatinine and beta-human chorionic gonadotrophin in the diagnosis of premature rupture of membranes. KAJOG 2011;2:41-7.
11. Midhet F. Prevalence and determinants of self-reported morbidity among pregnant women in rural areas of Pakistan. Int J Health Sci (Qassim) 2007;1:243-8.
12. Gibbs RS. Premature rupture of membranes. In: Scott JR, Gibbs RS, Karlan BY, Haney AF, Danforth DN, editors. Danforth's Obstetrics and Gynaecology. 9th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2003. p. 108-14.
13. Birkenmaier A, Ries JJ, Kuhle J, Burki N, Lapaire O, Hosli I. Placental alphamicroglobulin-1 to detect uncertain rupture of membranes in a European cohort of pregnancies. Arch Gynaecol Obstet 2012;1285:21-5.
14. Guibourdenche J, Luton D, Andre E, Noel M, Pourquet D. Rapid detection of insulin like growth factor-binding protein-1 and fetal fibronectin in cervico-vaginal secretions to diagnose premature membrane rupture. Ann Clin Biochem 1999;36:388-9.
15. Gaucherand P, Salle B, Sergeant P, Guibaud S, Brun J, Bizollon CA *et al*. Comparative study of three vaginal markers of premature rupture of fetal membranes; insulin-like growth factor binding protein-1, diamine oxidase pH. Acta Obstet Gynecol Scand 1997;76:536-40.
16. Zegels G, Van Raemdonck GA, Tjalma WA, Van Ostade XW. Use of cervicovaginal fluid for the identification of biomarkers for pathologies of the female genital tract. Proteome Sci 2010;8:1-23.
17. Carter RA, Pan K, Harville EW, McRitchie S, Sumner S. Metabolomics to reveal biomarkers and pathways of preterm birth: A systematic review and epidemiologic perspective. Metabolomics 2019;15:124.
18. Chandra H, Pahari S, Srivastava A, Masih L, Jamaluddin K, Barthwal CP. Is tertiary care treatment affordable to all? Explore alternative (s). Int J Health Sci (Qassim) 2009;3:187-92.
19. Araoye MO. Subjects selection. In: Research Methodology with Statistics for Health and Social Sciences. Ilorin: Nathadex Publishers; 2004. p. 115-29.
20. Adeniji AO, Atanda OO. Interventions and neonatal outcomes in patients with premature rupture of fetal membranes at beyond 34 weeks gestational age at a tertiary health facility in Nigeria. BJMMR 2013;3:1388-97.
21. Alsayegh AK, Roshdy S, Hany AA, Maha YS. Induction of labor with prostaglandin e2 in women with previous cesarean section and unfavorable cervix. Int J Health Sci (Qassim) 2007;1:211-6.
22. Aly SM. Risk of antimicrobial misuse. Int J Health Sci (Qassim) 2013;7:5-7.
23. Khan S, Khan AA. Study on preterm pre mature rupture of membrane with special reference to maternal and its fetal outcome. Int J Reprod Contracept Obstet Gynecol 2016;5:2768-74.
24. Moncla BJ, Pryke K, Rohan LC, Graebing PW. Degradation of naturally occurring and engineered antimicrobial peptides by proteases. Adv Biosci Biotechnol 2011;2:404-8.
25. Hanfy A. Urea and creatinine levels of washed vaginal fluid: A gold standard test to detect premature rupture of membranes. Med J Cairo Univ 2010;78:313-7.
26. Kariman N, Afrakhte M, Hedayati M, Fallahian M, Majd HA. Diagnosis of premature rupture of membranes by assessment of urea and creatinine in vaginal washing fluid. Iran J Reprod Med 2013;11:93-100.
27. Malchi F, Abedi P, Jahanfar S, Talebi F, Faal S, Zahedian M. Vaginal fluid urea and creatinine as indicators of premature rupture of membranes: A systematic review. Reprod Sci 2021;28:1-11.
28. Zanjani MS, Haghighi L. Vaginal fluid creatinine for the detection of premature rupture of membranes. J ObstetGynecol Res 2012;38:505-8.
29. Sekhavat L, Firouzabadi RD, Mojiri P. Practicability of vaginal washing fluid creatinine level in detecting premature rupture of membranes. Arch Gynaecol Obstet 2012;286:25-8.
30. Agbara JO, Fabamwo AO, Oshodi YA. Diagnosis of foetal membrane ruptures: Placental alpha-microglobulin-1 to the rescue. Trop J Obstet Gynaecol 2017;34:85-90.
31. Afshar B, Haseli A. The accuracy of sanitary pad of nitrazine test in the diagnosis of premature rupture of membranes. Int J Health Sci 2018;4:22-5.
32. Lamb EJ, Price CP. Kidney function tests. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. Philaldephia, PA: Saunders Elsevier; 2012. p. 680-6.
33. Greenberg N, Roberts WL, Bachmann LM, Wright EC, Dalton RN, Zakowski JJ *et al*. Specificity characteristics of 7 commercial creatinine measurement procedures by enzymatic and Jaffe method principles. Clin Chem 2012;58:391-401.