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The Study of Vaginal Fluid Urea, Creatinine, B-HCG and Placental Alpha-1 Microglobulin in Diagnosis of Premature Rupture of Membranes

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Abstract

Purpose: To evaluate and compare the reliability, accuracy and the cost benefit ratio of vaginal washing fluid urea, creatinine, Beta Human Chorionic Gonadotropin (β-HCG) and placental alpha Microglobulin-1 (PAMG-1) for diagnosis of premature rupture of membranes (PROM). **Material and Methods:** A diagnostic study conducted on 70 patients. The patients were divided into three groups: Group A (n = 25): (Confirmed PROM group) patients who were either in labor or not in labor, Gestational age was from 24 weeks onwards and fulfilled the following criteria and/or two of these criteria with low AFI positive pooling, positive nitrazine paper test, positive fern test. Group B (n = 25): (Suspected PROM group) patients who fulfilled the following criteria: Patients with fluid leakage complaint with negative pooling and/or negative nitrazine paper test and/or negative fern test. Group C (n = 25): (Control group with no PROM) patients that were admitted to prenatal clinic for their regular prenatal control visit with 24 - 42 weeks of gestational age without any complaint or complication and with negative pooling, negative nitrazine paper test and/or negative fern test. The vaginal washing fluid urea, creatinine, Beta-Human Chorionic Gonadotropin (β-HCG) and placental alpha Microglobulin-1 (PAMG-1) were determined for diagnosis of premature rupture of membranes (PROM). **Results:** PAMG-1 detection in cervico vaginal discharge was a very good test for diagnosis of PROM with high sensitivity, specificity, positive predictive value, negative predictive value, accuracy and P-value (96%, 100%, 100%, 95.84%, 97.78% and <0.0001 respectively). Urea and Creatinine is the second option in diagnosis of PROM with high sensitivity, and specificity after PAMG-1 with a privilege of low cost than PAMG-1. Furthermore they were more accurate than β-HCG. **Conclusion:** Detection of PAMG-1 in cervico vaginal discharge is promising

in diagnosis of PROM & especially in those cases of suspected PROM and it should be done as a worse trial in every case of suspected PROM. Urea and Creatinine is the second option in diagnosis of PROM with high sensitivity, and specificity after PAMG-1 with a privilege of low cost than PAMG-1. Also they were more accurate than β-HCG and they can be used if PAMG-1 is not available for detection of doubtful PROM cases.

Keywords

1. Introduction

Premature rupture of the membranes (PROM) refers to rupture of the fetal membranes prior to the onset of regular uterine contractions (before the onset of Labour). It complicates about 10% of pregnancies [1]. It may occur at term (≥37 weeks of gestation) or preterm (<37 weeks of gestation); the latter is designated preterm PROM (PPROM). Midtrimester PROM typically refers to PPROM at 16 to 26 weeks of gestation; this is an arbitrary definition, which varies slightly among investigators [2].

PROM is a clinical diagnosis actually. It is typically suggested by a history of watery vaginal discharge and is confirmed on sterile speculum examination [3]. The traditional minimally invasive gold standard for diagnosis of PROM relies on clinician's ability to document three clinical signs on sterile speculum examination: 1) Visual pooling of clear fluid in the posterior fornix of the vagina or leakage of the fluid from the cervical os; 2) An alkaline pH of the cervicovaginal discharge, which is typically demonstrated by nitrazine paper (whether the discharge changes nitrazine paper from yellow to blue); and/or 3) Microscopic ferning of the cervicovaginal discharge [4].

Diagnosis of PROM is easy in the presence of obvious rupture of membranes while several numbers of false positive and negative results obtained through applying conventional diagnostic methods in the suspected cases of PROM may result in inappropriate interventions such as hospitalization and induction of labor [5].

The accurate diagnosis of rupture of membranes can be difficult in obstetrics practice. The use of indigo carmine injection remains the diagnostic gold standard. However, it is too invasive to be used as routine practice. An ideal diagnostic tool should be noninvasive, able to detect ROM (sensitivity), exclude subclinical ROM (specificity), differentiate between amniotic fluid and other physiological fluids (cervicovaginal secretion, blood, and semen), and provide a rapid result (bedside test) [6]. With the exception of amniotic fluid being visualized directly from the cervical os, each of the available conventional standard diagnostic methods for diagnosing ROM has its own limitation [7]. Nitrazine test is to detect an alkaline pH in the amniotic fluid. Unfortunately, it has a high

false-positive rate as vaginitis, cervicitis, urine, blood, and semen or antiseptic agents may give rise to an alkaline PH [8]. The reported sensitivity of nitrazine test ranged from 90% to 97% with specificity from 16% to 70% [9]. Fern test gives a sensitivity and specificity of 51% and 70%, respectively, when patients were not in labour and increased to 98% and 88%, respectively, when used in patients in labour [10]. Visualization of crystallization of amniotic fluid on the slide may give false-positive result in the presence of semen and cervical mucus. On the other hand, contamination with blood or a dry swab as a result of technical error may lead to false-negative results [11].

Absence of an accurate non-invasive diagnostic test for ROM results in the emergence of various commercial tests using biochemical markers as indicator, e.g., fetal fibronectin, actim PROM (insulin-like growth factor binding protein-1 immunoassay), alpha-fetoprotein, and vaginal prolactin. Most of these biochemical markers failed to achieve an acceptable accuracy that is required in an ideal gold standard diagnostic test [12].

In recent years some studies have suggested measurement of Urea and creatinine in vaginal fluid for the diagnosis of ROM [13]. That study evaluates the reliability of urea and creatinine measurement for the diagnosis of ROM. They also reported the accuracy of urea and creatinine to determine the PROM from 90% - 100% [14] [15].

Urea plays an important role in the metabolism of nitrogen-containing compounds in the urine [16]. Creatinine is a break-down product of creatinine phosphate in muscles and is usually produced at a fairly constant rate and is mainly filtered out of the blood by kidneys. Urea and creatinine of fetal urine are the most important sources of amniotic fluid in second half of pregnancy [14]. Thus we hypothesized that vaginal fluid creatinine and urea may be helpful in diagnosis of PROM. Also, recently, a bedside immunoassay (AmniSure rapid immunoassay) has been used to detect fetal glycoprotein, placental alpha microglobulin-1 (PAMG-1) in cervico-vaginal secretions [17]. Placental alpha microglobulin-1 is considered an ideal substance to be used for detection of ROM. It has a concentration from 1000- to 10,000-fold higher in amniotic fluid than in the cervico-vaginal secretion (2000 - 25,000 ng/mL versus 0.05 - 2.0 ng/mL) [17] [18]. There is currently limited data available on the use of PAMG-1 immunoassay in clinical practice.

Another method for diagnosis of PROM is to detect B-HCG in cervico-vaginal secretions and has been studied for the evaluation of ROM. B-human chorionic gonadotropin is secreted by syncytiotrophoblasts and can be found in amniotic fluid as well as mother's blood or urine [19]. B-HCG has simplicity and ease of use as well as being cheaper compared to other substances which present in amniotic fluid [20].

2. The Aim of the Work

To evaluate and compare the reliability, accuracy and the cost benefit ratio of

vaginal washing fluid urea, creatinine, Beta-Human Chorionic Gonadotropin (β-HCG) and placental alpha Microglobulin-1 (PAMG-1) for diagnosis of premature rupture of membranes (PROM).

3. Patients and Methods

This was a prospective diagnostic study between October 2014 and April 2015 in Obstetrics and Gynecology Department at Tanta University Hospital. The study was approved by Tanta University hospital ethical committee. This study included 70 patients that had the following inclusion and exclusion criteria: Inclusion criteria: Pregnant women with gestational age from 24 weeks onwards who presented with symptom of rupture of membranes (ROM) (vaginal fluid leakage) either in labor or not. Exclusion criteria: pregnant women with active vaginal bleeding or infection, those diagnosed to have placenta previa, abruption and incidental hemorrhage (as vaginal examination is dangerous and any microscopic RBCs will affect the result), known fetal anomalies and any contaminated sample with urine, or blood due to abrasions during technique, and those with intrauterine fetal death.

3.1. Grouping

The patients were divided into three groups: Group A: (Confirmed PROM group) included 25 patients who fulfilled the following criteria and/or two of these criteria with low AFI: positive pooling, positive nitrazine paper test and positive fern test [9]. Group B: (Suspected PROM group) included 25 patients who fulfilled the following criteria: patients with fluid leakage complaint with negative pooling and/or negative nitrazine paper test and/or negative fern test. Group C: (Control group with no PROM) included 20 patients that were admitted to prenatal clinic for their regular prenatal control visit with 24 - 42 weeks of gestational age without any complaint or complication and with negative pooling, negative nitrazine paper test and/or negative fern test. All eligible pregnant women were informed regarding the study. Informed consent was taken from every patient. They were provided with verbal explanation for the purpose of the study, Privacy of participants, confidentially of the data and the method of sample collection. Any unexpected risks appeared during the course of the research were cleared to the participants and the ethical committee on time. Every participant had a code number. All pregnant women of the three groups were subjected to full history taking including personal history, the last menstrual period, amniotic fluid leakage (onset, amount, duration, color and odor of the fluid), history of amniotic fluid leakage in previous pregnancy, and past history of vaginal bleeding. General and abdominal examinations including fundal level, detect uterine contraction, and Auscultate fetal heart sound. We did trans-abdominal ultrasonography for gestational age, fetal viability, placental localization, congenital fetal malformation, and amniotic fluid index using 4 quadrant method. Gestational age was determined based on the first day of last menstruation pe-

riod in reliable cases, and ultrasound.

3.2. Sample Collection Techniques

Pregnant women were examined in lithotomy position, and sterile Cusco speculum was inserted under strict aseptic and antiseptic precautions. Pooling test: Inspection was done for leakage of fluid and result was registered as positive for leakage from posterior vaginal fornix, negative if there was no leakage detected [3] [4]. Nitrazine test: Then a sterile cotton tip applicator was inserted in deep vagina and was immediately transferred on nitrazine paper. A positive result was interpreted as a change of the nitrazine paper from orange to dark blue color which indicate that PH above 6.5 [21]. Fern test: A positive fern test is defined as visualization of arborisation or crystallization of amniotic fluid observed microscopically. At sterile speculum examination, after visualization for any presence of pooling of liquor, swab from posterior vaginal fornix or pooling site was performed with a sterile swab for fern test. The swab was smeared against a glass slide to create a very thin smear. The slide was then allowed to dry under room air for about 10 minutes without any heating. Finally, the slide was examined under a microscope (10 magnifications) for arborisation. Care was taken not to contaminate the slide with fingerprint, and technical errors such as dry swab were avoided. These steps were taken to reduce the false-positive and false-negative results in fern test [6].

Placental alpha-1 Microglobulin: Placental alpha microglobulin-1 protein assay (AmniSure) test was done using (AmniSure Kits®) with its instructions as follows, one visible line means a negative result for amniotic fluid, two visible lines is a positive result, no visible lines is an invalid result [11].

3.3. Vaginal Washing Fluid B-HCG Test, Urea and Creatinine Tests

Samples were collected as follows: in cases of pooling/flowing amniotic fluid, rinsing (injection) of 3 mL of sterile water into the posterior vaginal fornix then, aspiration of vaginal fluid with the same syringe was performed, while in the control group, initially 5 mL of sterile water was injected into the posterior vaginal fornix; thereafter, 3 mL was aspirated with the same syringe. After shaking the syringe and before sending it to the lab, 2 drops from the collected sample was applied on one step pregnancy test strip with a sensitivity of 25 mIU/ml and appearance of two lines was indicator for a positive result of the B-HCG test while one line was indicator for a negative results and no lines was indicator for invalid results (the results noted in the form of positive or negative.) [20]. Then, Samples were sent immediately to The Clinical Pathology Laboratory Department—Tanta University Hospital. Each sample at lab was centrifuged at 50 revolutions/second and the supernatant fluid was separated. Measurement of urea was performed by Enzymatic urease method while measurement of creatinine was performed by Rate Jaffé method. From the sample that collected, all speculum examinations were performed by the same obstetrician and all samples were studied in Clinical Pathology Laboratory, by the same technique and the same

technician in order to eliminate inter-observer sampling difference.

3.4. Statistical Methodology

Analysis of data was done by computer using Statistical Package for Science and Society (SPSS version 16) and Microsoft Excel 2010 Results.

4. Result

Table 1 shows that mean maternal age in confirmed, suspected and control groups were 25.45 ± 2.47 years, 24.87 ± 2.64 years and 24.84 ± 2.36 years respectively, the mean gestational age in confirmed, suspected and control groups were 34.04 ± 2.85 weeks, 33.91 ± 2.61 weeks and 33.79 ± 2.51 weeks respectively, the mean parity in confirmed, suspected and control groups were 1.13 ± 1.12, 1.29 ± 0.96 and 1.42 ± 1.17. There were no significant variations between mean age, gestational age and parity in three groups as P > 0.05.

Table 2 shows the comparison between the studied groups regarding previous history of PROM in previous pregnancy. There is no significant difference between the studied groups regarding previous history of PROM as (P-value > 0.05).

Table 3 shows the mean of Amniotic fluid index (AFI) between confirmed, suspected and control groups were 5.8 ± 1.22, 8.5 ± 2.36 and 13.03 ± 2.01 respectively. Also shown in (Error! Reference source not found.). There is significant difference between the studied groups regarding AFI as P-value < 0.05.

Table 1. Comparison between Confirmed, Suspected, and Control groups according to maternal age, gestational age and parity.

Variables	Group A Confirmed PROM		Group B Suspected PROM		Group C Control No PROM		P-value
	No. 25		No. 25		No. 20		
	Mean	±SD	Mean	±SD	Mean	±SD	
Maternal age (Years)	25.45	±2.47	24.87	±2.64	24.84	±2.36	0.43
Gestational age (weeks)	34.04	±2.85	33.91	±2.61	33.79	±2.51	0.86
Parity	1.13	±1.12	1.29	±0.96	1.42	±1.17	0.91

Table 2. Comparison between the studied groups as regarded previous history of PROM.

Variables		Group A Confirmed PROM		Group B Suspected PROM		Group C Control No PROM		Chi-square test	
		No. 25		No. 25		No. 20		X^2	P-value
		No.	%	No.	%	No.	%		
Previous history of PROM	Positive	4	16%	2	8%	1	5%	8.906	0.96
	Negative	21	84%	23	92%	19	95%		

Table 4: Sensitivity, Specificity, positive predictive value (PPV), Negative predictive value (NPV), accuracy and P-value of pooling, nitrazine and fern tests.

The accuracy of pooling, nitrazine and fern tests were 93.33%, 88.89% and 86.67% respectively. There is significant difference between confirmed, suspected and control group as P-value of pooling, Nitrazine and fern tests < 0.001 (Table 4).

Also, shown in (Figure 1). Pooling test has the highest specificity while fern test has the lowest specificity in this comparison between the three tests. Also pooling test has the highest PPV while nitrazine test has the highest NPV (Figure 1).

Table 5 shows that the sensitivity, specificity, positive predictive value, negative predictive value, accuracy and P-value of PAMG-1 and B-HCG. For PAMG-1 were 96%, 100%, 100%, 95.84%, 97.78% and <0.0001 respectively. For B-HCG were 88%, 90%, 91.67% 85.71%, 88.89% respectively. There was significant statistical difference between the studied groups as P-value < 0.05.

Table 6 shows that the mean results of vaginal fluid urea in the confirmed, suspected, and control groups were 0.76 ± 4.5, 7.41 ± 4.02 and 2.04 ± 1.01 respectively and the mean results of vaginal fluid creatinine in the confirmed, suspected, and control groups were 1.44 ± 0.45, 0.53 ± 0.33, and 0.25 ± 0.18 respectively. There was high significant difference between the studied groups regarding vaginal fluid urea concentrations (P-value < 0.001). Also, there is high significant difference between the studied groups regarding vaginal fluid Creatinine concentrations (P-value < 0.001).

Table 3. Comparison between the confirmed, Suspected and control groups as regarded amniotic fluid index (AFI).

AFI	Confirmed group	Suspected group	Control group	One way ANOVA	
	No. 25	No. 25	No. 20	F	P-value
Mean	5.8 cm	8.5 cm	13.03 cm	234.632	<0.0001
SD	±1.22	±2.36	±2.01		

Table 4. Comparison between the confirmed, Suspected and control group as regarded the results of pooling, nitrazine and ferning in diagnosis of PROM.

Variables	Sensitivity	Specificity	PPV	NPV	Accuracy	P-value
Pooling test	88	100	100	86.96	93.33%	<0.001
Nitrazine test	92	85	88.46	89.47	88.89%	<0.001
Fern test	92	80	85.19	86.89	86.67%	<0.001

Table 5. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and P-value of placental alpha-1 microglobulin and B-HCG.

Variables	Sensitivity	Specificity	PPV	NPV	Accuracy	P-value
PAMG-1 test	96%	100%	100%	95.84%	97.78%	<0.0001
B-HCG	88%	90%	91.67%	85.71%	88.89%	<0.0001

Table 6. Comparison of the mean results and P-value of urea and creatinine in vaginal washing fluids between the studied groups.

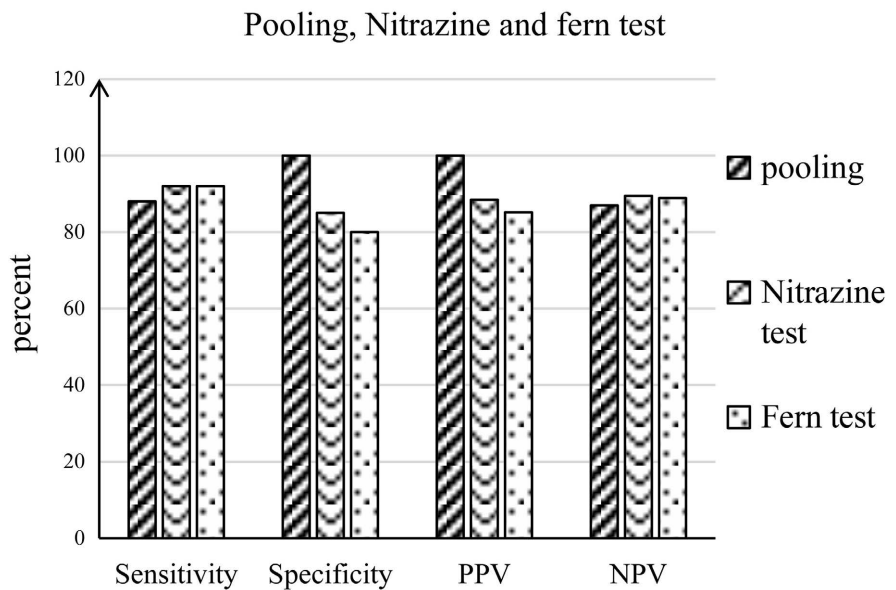| Variables	Group A Confirmed PROM		Group B Suspected PROM		Group C Control No PROM		One way ANNVA	
	Mean	±SD	Mean	±SD	Mean	±SD	F	P-value
Urea (mg/dL)	20.32	±5.6	7.41	±4.02	2.6	±3.06	44.379	<0.0001
Creatinine (mg/dL)	1.38	±0.56	0.53	±0.33	0.25	±0.18	33.586	<0.0001

Figure 1. Compare the resistivity, specificity, PPV, NPV of the pooling, nitrazine and fern test. Pooling test has the highest specificity while fern test has the lowest specificity. Also pooling test has the highest PPV while nitrazine test has the highest NPV.

Table 7 shows cut off value, sensitivity, specificity, positive predictive value, negative predictive value and accuracy in detecting PROM by evaluation of vaginal fluid urea and creatinine concentrations. Also shown in Figure 2 and Figure 3.

5. Discussion

PROM is associated with significant maternal and perinatal mortality and morbidity. Unfortunately, there is absence of an accurate and simple diagnostic tool to establish the diagnosis as the traditional way to diagnose ROM is subjective. The traditional "gold standard" relied heavily on the ability of the attending healthcare personnel to visualize pooling of liquor in the posterior vaginal fornix, detecting an alkaline vaginal pH, and observation of ferning effect from the liquor. However, each of these standard diagnostic methods was associated with high false positive or negative results. Several biochemical markers have been studied including fetal fibronectin, alpha-fetoprotein, and insulin like growth factor binding protein-1 to improve the accuracy of ROM detection. However, none had shown a promising result [6].

Table 7. Cut off value, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for Urea and Creatinine.

Variables	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Urea (mg/dL)	>4	98.8	96%	95%	96%	95%	95.56%
Creatinine (mg/dL)	>0.68	93.7	92%	100%	100%	90.91%	95.56%

Sensitivity and specificity / Urea

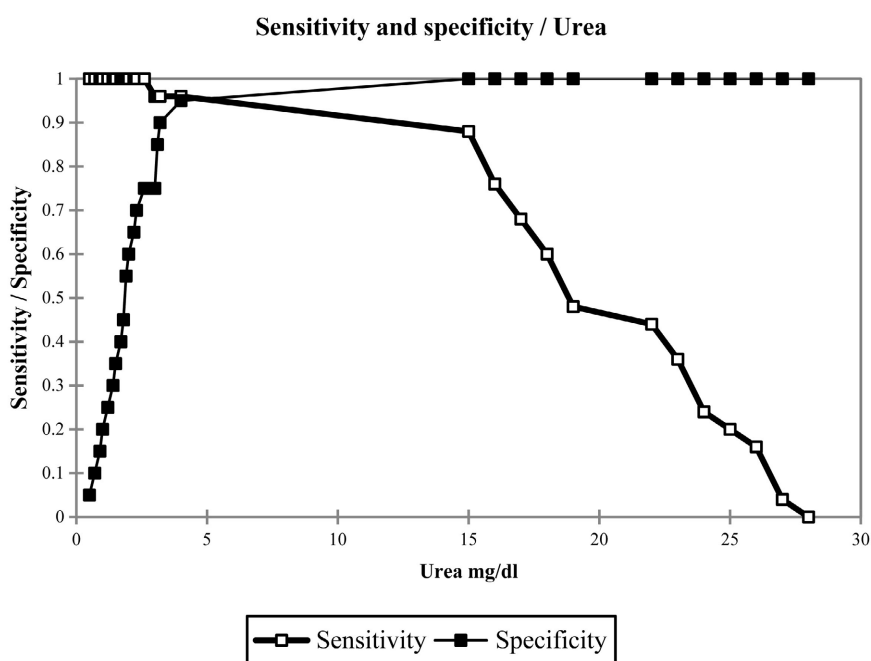**Figure 2.** Shows maximum sensitivity and specificity obtained at urea level 4 mg/dl.
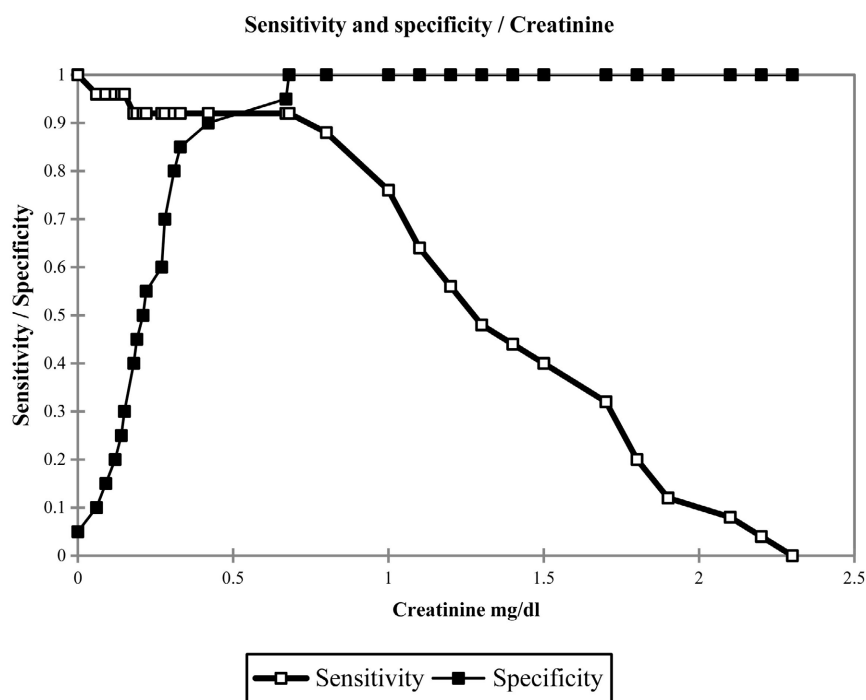
Sensitivity and specificity / Creatinine

Figure 3. Shows maximum sensitivity and specificity obtained at creatinine level 0.68 mg/dl.

A total of 70 pregnant women were included in the study, between 28 weeks to 39 weeks of gestation. They were divided into 3 groups: Group A—Confirmed PROM group; Group B—Suspected PROM group; Group C—Control group.

In this study the three groups were similar regarding baseline characteristics. There was no statistically significant difference between the three groups as regarded to the maternal age, gestational age and parity, making our results potentially more generalizable and enable us to be accurate in estimate which test can be used for diagnosis of premature rupture of membranes (PROM).

In the present study, there was highly statistically significant difference detected between the studied groups as regarded AFI (P-value < 0.0001). Both confirmed and suspected groups had a lower AFI compared to control group.

These results are in agreement with the study performed by Mohamed *et al.*, who reported that there was statistically significant difference between confirmed, suspected and control groups according to AFI [22]. Also, these results are consistent with the study performed by Bahasadri and by Erdemoglu who reported that there was statistically significant difference between the studied groups according to AFI as P-value < 0.001 [9] [20]. On the other hand, Kafali 2007 concluded that there was no significant statistical difference among the two groups regarding AFI [14].

The results of the present study also confirm the study conducted by Frigo *et al.*, 1998 who suggested that ultrasound examination is an important tool for the diagnosis of PROM [23]. On the other hand, Kafali who concluded that there was no significant statistical difference among the two groups regarding AFI. Although oligohydramnios without evident fetal urinary tract malformations or fetal growth restriction may be suggestive of membrane rupture, ultrasound alone cannot diagnose or exclude membrane rupture with certainty [14].

This study shows that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of pooling test were 88%, 100%, 100%, 86.96%, and 93.33% respectively. The p-value between confirmed and control groups was < 0.001 which show significant difference among the studied groups. This study also shows that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Nitrazine test were 92%, 85%, 88.46%, 89.47% and 88.89% respectively. Also shows the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Fern test were 92%, 80%, 85.19%, 86.89%, and 86.67% respectively. Pooling test has the highest specificity while fern test has the lowest specificity in comparison between the pooling, nitrazine, and fern tests. Also pooling test has the highest PPV while nitrazine test has the highest NPV. This result can be explained by that Ferning test has been associated with false-positive results due to contamination with fingerprints on a slide or contamination with semen or cervical mucus and false-negative results due to dry swabs or contamination with blood. Also Nitrazine evaluation has been associated with false positive results due to cervicitis, vaginitis, alkaline urine, blood, semen or antiseptics.

These results harmonize with those mentioned in the study performed by

Abdelazim and Makhlouf, 2012 who found that the sensitivity, specificity, PPV, NPV, and accuracy of Nitrazine test were 86.67%, 81.33%, 82.28%, 85.91%, and 84% respectively while fern test were 84%, 78.6%, 79.74%, 83.1%, and 81.33% respectively. They stated that Ferning has been associated with false-positive results in 5% - 30%; and false-negative results in 5% - 12.9%; Nitrazine evaluation has been associated with false positive results in 17.4%; and false negative results in 12.9% [11].

In this study, Nitrazine test showed a significant difference between the studied groups as P-value < 0.001. Also, Fern test showed a significant difference between the studied groups. (P < 0.001). These results of nitrazine and fern test consistent with the study performed by Bahasadri 2013 who found that Positive nitrazine test showed a significant difference between the three groups (P < 0.001) and had a sensitivity of 47% and specificity of 82.5%. Also, Positive fern test showed a significant difference between the three groups (P < 0.001) and had a sensitivity of 51.8% and specificity of 87.5% for detecting ROM [20].

As regard to B-HCG the present study shows that the sensitivity, specificity, PPV, NPV and accuracy of B-HCG were 88%, 90%, 91.67%, 85.71%, and 88.89% respectively. And also there was significant statistical difference between the studied groups (P-value < 0.0001). Mohamed *et al.* stated that the Sensitivity, specificity, PPV, NPV and accuracy were 94%, 86%, 93.1%, 87.8% and 91.3% as regarded to B-HCG. Also, it stated that there was very high significant difference between confirmed, suspected and control groups as regard B-HCG level in vaginal fluid (P-value < 0.001) [22]. In another study performed by Cooper 2004. The B-HCG test was positive in (79%) of the PPROM patients and in (3.6%) of the controls (sensitivity 79%, specificity 96%, PPV 95%, NPV 84%). They concluded that Qualitative HCG testing of cervico-vaginal washings appears to be an useful predictor of PPROM [24]. Kim *et al.*, 2005 performed a study on 120 pregnant women found the cut-off level of β-HCG to be 39.8 mIU/ml with a sensitivity, specificity, PPV, NPV 95.5%, 94.7%, 91.3% and 97.3% [5]. Other studies also showed higher cut off levels as they used the ROC curve analysis while in the present study, the normal bedside urine pregnancy test kit was used.

In a study performed by Mangano *et al.* 2000, it was performed on 52 women (20 women with intact membranes, 21 women with definitive ROM and 11 women with suspicious ROM), the researchers concluded that vaginal washing fluid B-HCG is a suitable, cheap and non-invasive method for the diagnosis of PPROM [25] [26]. In the other hand another study performed by Shahin and Raslan 2006 which evaluated vaginal fluid concentrations of three markers (AFP, prolactin and B-HCG), they were significantly higher in the PROM group than in the control group (p < 0.001). Receiver operator curve analysis indicated that AFP had higher specificity, sensitivity, positive and negative predictive values, and efficiency than the other two markers named prolactin and B-HCG. The specificity, sensitivity, positive and negative predictive values, and efficiency were 72%, 84%, 75.8% and 78% for B-hCG respectively [19].

In the present study, the mean vaginal fluid urea in confirmed, suspected and

control groups were 20.32 ± 5.6, 7.41 ± 4.02, 2.6 ± 3.06 respectively. The sensitivity, specificity, PPV, NPV and accuracy of washing vaginal fluid urea were 96%, 95%, 96%, 95%, and 95.56% respectively with cut off value > 4 mg/dl. There was statistically significant difference between the studied groups as regarded vaginal fluid urea (P-value < 0.001). The mean vaginal fluid creatinine in confirmed, suspected and control groups were 1.38 ± 0.56, 0.53 ± 0.33, 0.25 ± 0.18 respectively. The sensitivity, specificity, PPV, NPV and accuracy of washing vaginal fluid creatinine were 92%, 100%, 100%, 90.91%, and 95.56% respectively with cut off value > 0.68 mg/dl. There was statistically significant difference between the studied groups as regarded vaginal fluid creatinine (P-value < 0.001).

The results of the present study coincides with a study performed by Kariman *et al.* 2013 that was performed on 179 pregnant women divided into 3 groups (1—confirmed, 2—suspected, and 3—control groups) found that the mean vaginal fluid urea levels in group 1, 2 and 3 were 13.77± 5.41 mg/dl, 4.71 ± 3.64 mg/dl and 5.13 ± 5.97 mg/dl respectively, and the differences were statistically significant (p < 0.001). Furthermore, the mean vaginal fluid creatinine levels in group 1, 2 and 3 were 1.58 ± 1.01 mg/dl, 0.36 ± 0.23 mg/dl and 0.22 ± 0.10 mg/dl respectively. The differences between groups were statistically significant (p < 0.001). The cut off value of washing vaginal fluid urea was 6 mg/dl while the cut off value of creatinine was 0.45 mg/dl [21]. In another study conducted by Kafali and Oksüzler, 2007 that was performed on 139 pregnant women divided into 3 groups showed a statistically significant difference among all groups regarding urea and creatinine levels [14].

In a study conducted by Osman 2014, they found that the sensitivity, specificity, positive and negative predictive values and accuracy for urea and creatinine were all 100% and for qualitative β-hCG 83%, 100%, 100%, 85.6%, and 91% respectively [26]. So the net results of our study harmonize with Osman study that B-HCG was less accurate than urea and creatine as B-HCG had lower sensitivity, specificity, PPV, NPV and accuracy comparing with vaginal fluid urea and creatinine. The same result is concluded by Gurbuz 2004 that vaginal fluid creatinine is an extremely useful marker in doubtful cases of PROM. They concluded that the creatinine assay is cheaper and faster than other methods, and has higher sensitivity and specificity to establish accurate diagnosis [13].

Indeed Lee *et al.*, 2007 stated that the current diagnostic methods use nitrazine/pH, assessment of pooling, and microscopic ferning testing lack reliability and become progressively less accurate with passage of time since membrane rupture. In cases of prolonged PROM, these tests provide no better diagnostic information than that obtained by simple clinical evaluation [27]. He performed a prospective observational study in consecutive patients with signs or symptoms of rupture membranes on a total 183 patients, 157 (87%) had rupture of membranes at their initial presentations using the same gold standard that have been used in the present study. Placental alpha-microglobulin-1 immunoassay proved to have a sensitivity of 98.7%, specificity of 87.5%, positive predictive value of 98.1%, and negative predictive value of 91.3%. A false-positive

test (defined as a positive placental immunoassay in women who were subsequently determined not to have ROM was documented in three cases. So he concluded that the placental alpha-microglobulin-1 immunoassay is a rapid and accurate method for confirming the diagnosis of ROM. Moreover, its performance appears to be superior to conventional clinical assessment (pooling, nitrazine, ferning) and the nitrazine test alone [27].

In this study, the sensitivity, specificity, PPV, NPV and accuracy of detection of placental alpha microglobulin-1 (PAMG-1) were 96%, 100%, 100%, 95.84%, and 97.78% respectively.

Eleje 2015 state that ROM was diagnosed if two out of three methods from standard clinical assessment (pooling, positive nitrazine test or ferning) were present. They also conclude that accuracy, sensitivity and specificity for the PAMG-1 test were 97.2%, 97.4% and 96.7%, higher than for standard clinical assessment (SCA) which were 83.7%, 87.9% and 70.5%, respectively (P < 0.001). In women without pooling, accuracy of the PAMG-1 test was 96.7%, while it was 40.0% with standard clinical assessment (SCA) [28]. These results are consistent with those reported by Cousins [18], Sosa and Abdelazim. Sosa concluded that the PAMG-1 immunoassay in vaginal fluid yielded results that were comparable to those of the instillation of indigo carmine into the amniotic cavity; therefore, propose that PAMG-1 is a sensitive and specific test to assess ROM in patients with an equivocal diagnosis based on simple tests [29]. Also Abdelazim reported The sensitivity and specificity of PAMG-1 to diagnose PROM were 97.33% and 98.67%, respectively, compared with 84% sensitivity and 78.67% specificity for Ferning test and 86.67% sensitivity and 81.33% specificity for Nitrazine test. The positive predictive value (PPV) and negative predictive value (NPV) of PAMG-1 were 98.64 and 97.37%, respectively, compared with 79.74% PPV and 83.1% NPV for Ferning test and 82.28% PPV and 85.91% NPV for Nitrazine test. PAMG-1 was accurate (98%) for detection of PROM than Ferning (81.33%) or Nitrazine (84.0%) tests [11].

6. Conclusions

Detection of PAMG-1 in cervicovaginal discharge is promising in diagnosis of PROM & especially in those cases of suspected PROM as it was very valuable with high sensitivity, specificity, PPV, NPV, accuracy and easy use as mentioned previously. Although the cost of the test is high in comparison with other diagnostic tools and in comparison with detection of urea, creatinine and B-HCG, the high accuracy and reliability of it is a great benefit.

Urea and Creatinine is the second option in diagnosis of PROM with high sensitivity, and specificity after PAMG-1 with a privilege of low cost than PAMG-1. Furthermore they were more accurate than β-HCG and they can be used if PAMG-1 is not available for detection of doubtful PROM cases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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