

DIAGNOSTIC ACCURACY OF B-HCG AND NITRAZINE PAPER TEST IN VAGINAL WASHINGS TAKING AMNIOTIC FLUID POOLING AS GOLD STANDARD OF DIAGNOSING PREMATURE RUPTURE OF MEMBRANES

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ABSTRACT

Background: Premature Rupture of Membranes (PROM) is common in obstetrics and management of such patients depends on whether the rupture has occurred or not. With membranes ruptured the fetus is deprived of protection provided within the amniotic cavity. Beta-Human chorionic gonadotrophin (β -HCG) is a hormone and is present in high concentration in amniotic fluid as well as in the blood and urine of the mother and is studied as possible predictor of preterm labour and as marker of PROM.

Objectives: To compare the diagnostic accuracy of B-hCG & nitrazine paper test in vaginal washings taking amniotic fluid pooling as gold standard for diagnosing premature rupture of membranes.

Methods: It was a comparative cross-sectional study conducted at Unit Department, of Obstetrics & Gynecology, Lahore General Hospital, Lahore. After that vaginal washings were taken for β -hCG testing. A pregnancy test kit (Accu Check) was used for detection of β -hCG in vaginal fluid washings. According to amount of μ -hCG in the washings the result has been positive as early as 40 Seconds but for labelling the result negative 5 minutes complete reaction time has been observed. On Nitrazine kit, the positive test is indicated by distinct colour band on both; control and test side.

Results: Mean age of women was 27.17 ± 4.55 years. Mean age of gestation was 36.16 ± 3.30 weeks. Sensitivity & specificity of Nitrazine Paper Test were 92.17% & 66.67%. However, the PPV & NPV for Nitrazine Paper Test was 98.15% & 30.77% respectively. Sensitivity & specificity of β -hCG Test was 94.35% & 75%. However, the PPV & NPV for β -hCG test was 98.64% & 40.91% respectively.

Conclusion: Results of this study showed that B-subunit of hCG measured by over-the-counter available pregnancy test kit is a dependable quick and easy test for detection of premature rupture of membranes. This test can be performed on the bed side of the patient without Lab involvement. This test can be promoted as an additional help for the diagnosis of doubtful and ambiguous cases of premature rupture of membranes.

Keywords: Preterm Rupture of Membranes, beta-Human chorionic gonadotrophin, Nitrazine paper test, Vaginal Washings, Amniotic fluid pooling

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INTRODUCTION

Premature Rupture of Membranes (PROM) is defined as rupture of chorioamniotic membranes before the onset of labour at any stage of pregnancy. Mostly it occurs near term or it may happen as unexpected complication before 37 weeks of gestation in which case it is termed as

preterm PROM.¹⁻² The distinction between PROM and Premature PROM is important as etiology of PROM (physiological process) differs from Premature PROM (pathological process). The basic underlying mechanism of membrane rupture is reduction in membrane strength due to programmed cell decay, activation of catabolic enzymes like collagenase as well as mechanical forces.³⁻⁴

PROM occurs in proximately 1-3% of pregnancies and is responsible for 1/3 of all the preterm birth. PROM has been accompanied with risks for mother, fetus and neonate.⁵ Large number of exogenous risks factors has been related to PROM, like infection in genital tract, cervical incompetence, nutritional deficiency e.g. ascorbic acid and copper.⁶

Patients having PROM presents with gush of fluid from vagina, may be accompanied by bleeding per vagina, perineal heaviness but not with contractions. It leads to severe hazardous consequences to pregnant females like chorioamnionitis, preterm labour, abruptio placental, and post-partum endometritis, especially complications associated with prolonged conservative management of Premature PROM occurring at earlier gestation ending in evacuation and curettage, maternal sepsis and even maternal death.⁷

Clinically spontaneous rupture of membranes presents with a history of involuntary loss of gush of watery fluid from vagina which soaks under garments and smells like amniotic fluid. Patient's history and inspection of amniotic fluid leakage from cervix on sterile speculum evaluation can lead to more than 90% cases of PROM, particularly when large quantity of amniotic fluid is leaking, nevertheless on different occasions the leak might be very less in quantity or irregular and membranes may rupture without symptoms.⁸

In patients having negative speculum examination or ambiguous history to confirm or deny PROM traditionally used tests are, nitrazine blue test (based on alkaline pH of vaginal fluid which turns nitrazine yellow paper to blue paper test), fern test (demonstrates ferning pattern of vaginal fluid secretions after being dried out) and dye test (which involves administration of dye into amniotic fluid trans abdominally and observing blue staining in vaginal fluid after 24 hours.⁹⁻¹¹

Recently studies have been carried out to develop a specific, effortless, non-invasive and authentic test for the detection of PROM. Various biochemical agents had been evaluated with varying results.^{12, 13} Clinically nearly 10% of cases of suspected PROM needs further evaluation especially in outpatients. Different bedside test known as point-of-care testing - POCT as frequently used in current practice. As it does not involve laboratory, specific instruments or trained person. Human chorionic gonadotrophin (hCG) in vaginal secretions studied by ELISA method has given varied results for detection of PROM.¹⁴ Cooper et al., & Kariman et al., were the researchers who utilized maternal urine one step dipstick

method with a cut-off of 25 mIU/ml for detection of PROM.^{15, 16}

So one step B-hCG dipstick is a simple, quick easily available and non-invasive method for diagnosis of pregnancy utilized by most of health personnel in both emergency and outpatient settings. Traditionally, B-hCG is detected in amniotic fluid collection by using laboratory radio immunoassay but this is a time-consuming method. Through this study, we aimed to develop simple, quick, easy and a reliable testing method to diagnose PROM, which must not need laboratory testing, provides quick and fast output and must be cost-effective. The objective of this study was to compare accuracy of β HCG & nitrazine paper test in vaginal washings taking amniotic fluid pooling as Gold standard in females with suspicion of premature rupture of membranes (PROM).

METHODS

This cross-sectional study was conducted at Department of Obstetrics & Gynaecology PGMI / Lahore General Hospital Lahore from August 2014 to August 2015. Total 242 patients were included by using confidence level at 95%, margin of error as 5% and anticipated population proportion i.e. 19.53%.¹⁷ Patients were enrolled by applying non-probability, consecutive sampling. Females of age 18 to 40 years, presented at gestational age ≥ 28 weeks (calculated by LMP / earlier scan) complaining of per vaginal leaking, both primigravida and multigravida with alive singleton pregnancy with no congenital anomaly (confirmed on USG) were included in the study. Patients with vaginal bleeding -- spontaneous or traumatic, Low-lying placenta on (USG) or with chorioamnionitis were excluded

After approval from ethical board, 242 patients fulfilled inclusion and exclusion criteria, attending Emergency and Labor ward of LGH, Lahore were enrolled in this research. Written informed consent was taken from every pregnant female enrolled in this study after explaining the purpose of study. Their demographic profile (name, age, address, gestational age and parity) was recorded. After that B-hCG vaginal fluid sampling done as under: Sterilized Cusco's speculum was inserted to visualize the cervix. The amniotic fluid pooling was observed in the posterior fornix and a nitrazine paper test was done. Few inches of Nitrazine paper were used for the test. Paper was secured in the holder before tearing. Vaginal secretions were touched with the paper. The color on the strip of paper was immediately matched with the closest color on the chart in the dispenser. Each color corresponds to a specific pH. The normal pH for amniotic fluid, normal vaginal secretions and urine is 7 to 7.5, 4.5 to 5.5 and 5.5 to 6.5 respectively. The interpretation of Nitrazine test was done as shown in the table below:

Membranes Probably Intact

Yellow	pH 5.0
Olive Yellow	pH 55
Olive Green	pH 60
Ruptured Membranes	
Blue Green	pH65
Blue Gray	pH 7.0
Deep Blue	pH75

In next step, the following method was adopted to collect vaginal washings:

Vaginal fluid was mopped out with sterilized gauze. The posterior vaginal fornix was irrigated with 5 ml of sterile saline solution and 3 ml it was sucked out with the same syringe. A commercial pregnancy test kit (Accu-Chek) was used for detection of β-hCG in vaginal fluid washings. The test was considered positive at threshold value of 36mIU/ml of B-hCG which is greater than the level of B-hCG present in cervico-vaginal secretion. The test may give a positive result as early as 40 second on the basis of B-hCG concentration. Nevertheless negative result was only labelled after observing the complete reaction time of 5 minutes. The negative test was indicated by only one colour band on test side and excludes amniotic fluid. The positive test was indicated by distinct colour band on both; control and test region. The invalid test was indicated by no visible band; or one colour band appears on control side. Finally, the test was done again with new kit. The data generated with nitrazine paper test and B-hCG pregnancy test kit is compared statistically with amniotic fluid pooling. All the information related to study procedure was recorded on a Proforma.

Data was analyze by using SPSS a 20.0. 2x2 tables were generated to calculate sensitivity specificity, PPV and NPV and diagnostic accuracy of β-hCG and nitrazine paper test keeping amniotic fluid pooling as gold standard.

RESULTS

The mean age of females was 27.17 ± 4.55 years. Females presented on average mean gestational age of 36.16 ± 3.30 weeks. On Nitrazine, there were 216 (89.3%) females who were detected as positive while 26 (10.7%) were detected as negative. On B-hCG, there were 220 (90.9%) females who were detected as positive while 22 (9.1%) were detected as negative. On Amniotic fluid pooling, there were 230 (95.0%) females who were detected as positive while 12 (5.0%) were detected as negative. Table 1

Table 2 shows diagnostic accuracy of Nitrazine Paper Test. Sensitivity and specificity of Nitrazine Paper Test was 92.17% and 66.67%. However, the positive predictive value and negative predictive value for Nitrazine Paper Test was 98.15% and 30.77%

respectively. The overall diagnostic accuracy of this test is 90.91%.

Table 3 shows B diagnostic accuracy of β-hCG. Sensitivity & specificity were 94.35% and 75%. However, the PPV & NPV for β-hCG Test was 98.64% and 40.91% respectively. The overall diagnostic accuracy of this test is 93.39%.

Table 4 shows a comparison between various diagnostic indices of Nitrazine paper test vs. B-hCG Pregnancy kit Test. Sensitivity of Nitrazine test vs. B-hCG test was 92.1% and 94.35% respectively while specificity of Nitrazine test vs. B- hCG test was 66.67% and 75% respectively. Nitrazine paper test had 90.91% DA as compared to93.39%obtained for B-hCG test.

Table 1: Demographics of females included in the study

N	242
Age (year)	27.17 + 4.55
Gestational age	36.16 +3.30
Nitrazine	
Positive	216 (89.3%)
Negative	26 (10.7%)
B-HCG	
Positive	220 (90.9%)
Negative	22 (9.1%)
Amniotic fluid pooling	
Positive	230 (95%)
Negative	12 (5%)

Table 2: Accuracy of Nitrazine paper test taking amniotic fluid pooling as gold Standard

Nitrazine paper test	Amniotic Fluid Pooling		Total
	Positive	Negative	
Positive	212 (92.2%)	4 (33.3%)	216
Negative	18 (7.8%)	8 (66.7%)	26
Total	230	12	242

Sensitivity=92.17%, Specificity=66.67%, PPV=98.15%, NPV=30.77% and Diagnostic Accuracy 90.91%

Table 3: Accuracy of Beta-hCG taking amniotic fluid pooling as gold standard

B-hCG	Amniotic Fluid Pooling		Total
	Positive	Negative	
Positive	217 (94.3%)	3 (25%)	220
Negative	13 (5.7%)	9 (75%)	22
Total	230	12	242

Sensitivity=94.35%, Specificity=75%, PPV=98.64%, NPV=40.91% and Diagnostic Accuracy=93.39%

Table 4: Comparison of diagnostic indices of nitrazine paper test and B-hCG pregnancy kit test

	Nitrazine Paper Test	β -hCG Pregnancy Kit Test
Sensitivity	92.17%	94.35%
Specificity	66.67%	75.0%
PPV	98.15%	98.64%
NPV	30.77%	40.91
Diagnostic accuracy	90.91%	93.39%

DISCUSSION

Premature rupture of membranes (PROM) is diagnosed clinically as suggested by gush of watery vaginal discharge is confirmed by visualization of amniotic fluid on sterile speculum examination.⁷ Traditionally the gold standard for detection of PROM depend on finding 3 clinical signs) on per speculum examination by the obstetrician: 1) Visualization of amniotic fluid pooling in the posterior fornix of the vagina or leakage of clear fluid from external cervical OS; 2) An alkaline pH of the cervico-vaginal secretions revealed by nitrazine paper test (where the nitrazine paper changes color from yellow to blue by alkaline pH of cervico-vagina secretions. 3) Ferning pattern shown by cervico-vaginal secretions after drying out under microscopy.^{7, 13, 18}

Early or proper detection of premature rupture of membranes is of much significance as most of relative obstetric complications like, chorioamnionitis and preterm delivery can be avoided or otherwise false diagnosis can result in unwanted interventions like hospitalization, administration of antibiotics and corticosteroids as well as induction of labour.^{19, 20}

As much as 90% of cases can be diagnosed clinically on the basis of history, finding of reduced amniotic fluid index on ultrasonography, visualization of amniotic fluid coming from cervix and or positive nitrazine and fern test on vaginal secretions. Various biological markers have been evaluated to diagnose amniotic fluid but as yet none has been proven to be gold standard for detection of premature rupture of membranes. Nitrazine paper test can give a false positive result due to contamination with blood, seminal fluid, antiseptic solutions and also in the presence of bacterial vaginosis. The fern test can also give false positive results which can be avoided by taking sample from posterior fornix or lateral vaginal wall.^{21, 22} Similarly false negative fern test can be obtained in case of prolonged leaking resulting in reduction in amniotic fluid. In such cases and patients having strong clinical suspicion of membrane rupture, patient is advised to take rest in recumbent position or alternatively other measures are taken.²²

As there are restrictions with existing gold standard method for detection of PROM (namely, amniotic fluid visualization, nitrazine paper test and fern test),

researchers are looking for more authentic tests. These tests relied on presence of certain biochemical markers in the cervico-vaginal secretions after membrane rupture absent in women with intact membranes. Various biological markers studied so far include alpha-fetoprotein,²³ fetal fibronectin,²⁴ insulin like growth factor binding protein 1,²⁴ prolactin,²⁵ beta-subunit of human chorionic gonadotrophin (β -hCG), creatinine, urea, lactate, and placental alpha-microglobulin 1.²⁶

Varying results have been obtained by using such tests. Although recently proteomic technology by finding novel protein biomarkers in the cervico-vaginal secretions have been used for women having intramniotic infection or who have preterm delivery but not used for detection of preterm PROM.^{7, 8, 13}

In the last few decades various diagnostic methods have been developed and investigated which utilized the presence of these biochemical markers in the amniotic fluid. These tests have strong predictive value for detection of PROM as concentration of the biochemical markers in the amniotic fluid is much higher than in the vagina secretions. Various studies have proved the superiority of these tests due to greater ease of sample processing and accuracy than the conventional tests.²⁶

Kariman from Iran compared enzyme linked immunosorbent assay and three quick dipstick methods for detection of PROM. The findings showed that sensitivity, specificity, PPV, NPV & diagnostic accuracy of ACON kit for diagnosis of PROM, based on beta-hCG level were 90%, 92%, 92.1%, 90% and 91% respectively.²⁷ Findings of our study is agreed with findings of study done by Kariman.

In another study sensitivity, specificity, PPV & NPV of Beta-hCG for diagnosis of PROM was reported as 100%, 100% 85.6% & 91% respectively.²⁸ This study also support the findings of this study showing high sensitivity and PPV for B-hCG test.

Osman and Elghazaly reported in their study that the sensitivity & specificity of beta-hCG were 83% and 100%, while PPV & NPV were 100% & 85.6%, and overall accuracy was 91%.²⁹ Marcedian Lin in his study compared two bedside tests for detection of PROM by using presence of insulin-like growth factor-binding protein-I and placental α -microglobulin-1 in cervico-vaginal secretions. Both studies showed similar results for detection of PROM.³⁰ Ibrahim A. Abdelazim in his study proved that Actim PROM test by using insulin-like growth factor-binding protein-1 is simple bedside test used as complementary test to confirm the PROM.³¹

Studies which measured vaginal fluid B-hCG has accuracy lower than placental α -microglobulin-1.

Measuring the beta subunit of hCG is a simple and can be done in most of the centres even in emergency situations were as measuring the levels of other biological markers is more costly and not feasible to do in each and every center around the clock. In the end it is concluded that as

level of beta subunit of hCG is much elevated in patients with membrane rupture as compared with those with intact membranes it is utilized as rapid proper dependable method of diagnosing PROM. As laboratory facility of B-hCG measurement is available in most of the centres it can also be used for diagnosing PROM alternatively.

Limitations associated with these tests are, nitrazine blue test as poor specificity and false positive test is reported in the presence of blood, semen, lubricants and antiseptic solutions, similarly smear taken from cervix for fern test may also give a false positive result and dye test is an invasive test and sometimes may itself cause rupture of membranes.³² Alternatively detection of biomarkers present in amniotic fluid in high concentrations seen after membrane rupture, either not present or exist in very small concentration in cervico-vaginal secretions during pregnancy with intact amniotic membranes has been studied for confirmation of PROM.²⁶

Beta-HCG is a glycoprotein hormone and is secreted by placental syncytiotrophoblast during pregnancy. It is present in high concentrations in the amniotic fluid as well as maternal blood and urine. Beta-hCG is studied recently as a predictor of preterm labour and a marker for premature rupture of membranes. Judgment of membrane rupture can also be done by dipstick test on vaginal fluid or discharge like Actim Prom or Ammnisure.³³

Smaller sample size is the limitation of this study.

CONCLUSION

Results of this study showed that B-subunit of hCG measured by over-the-counter available pregnancy test kit is a dependable, quick and easy test for detection of PROM. This test can be performed on the bed side of the patient without Lab involvement. The test should be promoted as an additional help for the diagnosis of doubtful and ambiguous cases of premature rupture of membranes.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board of Postgraduate Medical Institute / Ameer- ud-Din Medical College/ Lahore General hospital, Lahore.

REFERENCES

1. Smith C, Greenspoon J, Phelan J, Platt L. Clinical utility of the nonstress test in the conservative management of women with preterm spontaneous premature rupture of the membranes. *The Journal of reproductive medicine*. 1987;32(1):1-4.
2. Ebisutani K, Wang CK, Ahn HJ, Broady AJ, Kaneshiro B. Utility of Routine Testing for Chlamydia and Gonorrhoea in the Setting of Preterm Delivery or Premature Preterm Rupture of Membranes. *Hawai'i Journal of Health & Social Welfare*. 2021;80(6).
3. Kacerovsky M, Pliskova L, Bolehovska R, Lesko D, Gerychova R, Janku P, et al. Cervical Gardnerella vaginalis in women with preterm prelabour rupture of membranes. *Plos one*. 2021;16(1):e0245937.
4. Vajrychová M, Stráník J, Pimková K, Barman M, Kukla R, Zedníková P, et al. Comprehensive proteomic investigation of infectious and inflammatory changes in late preterm prelabour rupture of membranes. *Scientific reports*. 2020;10(1):1-14.
5. Galaz J, Romero R, Slutsky R, Xu Y, Motomura K, Para R, et al. Cellular immune responses in amniotic fluid of women with preterm prelabour rupture of membranes. *Journal of perinatal medicine*. 2020;48(3):222-233.
6. Jain S, Jaiswar S, Singh N, Deo S, Agarwal M, Ali W. Beta-HCG Concentration in Vaginal Fluid: Used as a Diagnostic Biochemical Marker for Preterm Premature Rupture of Membrane in Suspected Cases and Its Correlation with Onset of Labour. *The Journal of Obstetrics and Gynecology of India*. 2020;70:283-288.
7. Caughey AB, Robinson JN, Norwitz ER. Contemporary diagnosis and management of preterm premature rupture of membranes. *Reviews in obstetrics and gynecology*. 2008;1(1):11.
8. Zanjani MS, Haghighi L. Vaginal fluid creatinine for the detection of premature rupture of membranes. *Journal of Obstetrics and Gynaecology Research*. 2012;38(3):505-508.
9. Shimoya K. *Premature Rupture of Membranes*. *Preterm Labor and Delivery*: Springer; 2020. p. 207-212.
10. Swiatkowska-Freund M, Traczyk-Łos A, Partyka A, Obara K, Damdinsuren A, Preis K. Perinatal outcome in preterm premature rupture of membranes before 37 weeks of gestation. *Ginekologia polska*. 2019;90(11):645-650.
11. Shimoya K. *Premature Rupture of Membranes*. *Preterm Labor and Delivery*. 2019:207.
12. Knapik D, Olejek A. Analysis of cervicovaginal fluid in the diagnosis of premature rupture of membranes. *Ginekologia polska*. 2011;82(1).
13. Caughey AB, Urato AC, Lee KA, Thiet M-P, Washington AE, Laros Jr RK. Time of delivery and neonatal morbidity and mortality. *American journal of obstetrics and gynecology*. 2008;199(5):496. e1-. e5.
14. Dartibale CB, Uchimura NS, Nery L, Schumeish AP, Uchimura LYT, Santana RG, et al. Qualitative determination of human chorionic gonadotropin in vaginal washings for the early diagnosis of premature rupture of fetal membranes. *Revista Brasileira de Ginecologia e Obstetrícia*. 2017;39:317-321.
15. Cooper AL, Vermillion ST, Soper DE. Qualitative human chorionic gonadotropin testing of cervicovaginal washings for the detection of preterm premature rupture of membranes. *American journal of obstetrics and gynecology*. 2004;191(2):593-596.
16. 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. *Iranian journal of reproductive medicine*. 2013;11(2):93.

17. Liu J, Feng Z-C, Wu J. The incidence rate of premature rupture of membranes and its influence on fetal–neonatal health: A Report from Mainland China. *Journal of tropical pediatrics*. 2010;56(1):36-42.
18. Park JS, Lee S, Norwitz E. Non-invasive testing for rupture of the fetal membranes. *US Obstet Gynecol*. 2007;1:13-16.
19. Kim Y-H, Park Y-W, Kwon H-S, Kwon J-Y, Kim B-J. Vaginal fluid β -human chorionic gonadotropin level in the diagnosis of premature rupture of membranes. *Acta obstetrica et gynecologica Scandinavica*. 2005;84(8):802-805.
20. Esim E, Turan C, Unal O, Dansuk R, Cengizglu B. Diagnosis of premature rupture of membranes by identification of β -HCG in vaginal washing fluid. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2003;107(1):37-40.
21. Gurbuz A, Karateke A, Kabaca C. Vaginal fluid creatinine in premature rupture of membranes. *International journal of gynaecology and obstetrics*. 2004;85(3):270-271.
22. Kafali H, Öksüzler C. Vaginal fluid urea and creatinine in diagnosis of premature rupture of membranes. *Archives of gynecology and obstetrics*. 2007;275(3): 157-160.
23. Shahin M, Raslan H. Comparative study of three amniotic fluid markers in premature rupture of membranes: prolactin, beta subunit of human chorionic gonadotropin, and alpha-fetoprotein. *Gynecologic and obstetric investigation*. 2007;63(4):195-199.
24. Jeurgens-Borst AJ, Bekkers RL, Sporcken JM, van den Berg PP. Use of insulin like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2002;102(1):11-14.
25. Buyukbayrak E, Turan C, Unal O, Dansuk R, Cengizoglu B. Diagnostic power of the vaginal washing-fluid prolactin assay as an alternative method for the diagnosis of premature rupture of membranes. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2004;15(2):120-125.
26. Erdemoglu E, Mungan T. Significance of detecting insulin-like growth factor binding protein-1 in cervicovaginal secretions: comparison with nitrazine test and amniotic fluid volume assessment. *Acta obstetrica et gynecologica Scandinavica*. 2004;83(7): 622-626.
27. Kariman N, Hedayati M, Taheri Z, Fallahian M, Salehpoor S, Majd SA. Comparison of ELISA and three rapid HCG dipsticks in diagnosis of premature rupture of membranes. *Iranian Red Crescent Medical Journal*. 2011;13(6):415.
28. Mohamed A, Mostafa W. The Value of Measurement of Vaginal Fluid Urea, Creatinine & Beta HCG in the Diagnosis of Premature Rupture of Membranes. *KAJOG*. 2011;2:41-47.
29. Osman OM, Elghazaly M. Can vaginal washing fluid urea, creatinine and qualitative β -hCG diagnose suspected premature rupture of membranes? *Open Journal of Obstetrics and Gynecology*. 2014;4(15):967.
30. Palacio M, Kühnert M, Berger R, Larios CL, Marcellin L. Meta-analysis of studies on biochemical marker tests for the diagnosis of premature rupture of membranes: comparison of performance indexes. *BMC pregnancy and childbirth*. 2014;14(1):1-12.
31. Abdelazim IA, Abu-Faza M. Fetal Fibronectin (Quick Check fFn Test) Versus Insulin-Like Growth Factor Binding Protein-1 (Actim PROM Test) for De-tection of Premature Rupture of Fetal Membranes.
32. Baby HA, Begum R. Preterm Premature Rupture Membrane. *Patient Management in Obstetrics & Gynecology*. 2018:53.
33. Waters TP, Mercer B. Preterm PROM: prediction, prevention, principles. *Clinical obstetrics and gynecology*. 2011;54(2):307-312.

AUTHOR'S CONTRIBUTIONS

SI: Manuscript writing, Data collection, Statistical analysis

MAFZ: Supervision

MI: Data collection, Statistical analysis

MJ, FA: Data collection