AmniSure: A Point of Care Diagnostic for Preterm, Term Prelabor Rupture of Membranes

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INTRODUCTION

Prelabor rupture of membranes (PROM) at term occurs in 10% births, preterm prelabor rupture of membranes (PPROM) in 2-3.5% births.[1-3] Rapid diagnosis, appropriate management are essential for best pregnancy outcome.[4,5] Therefore, timely, accurate diagnosis is essential.

Three commonly used methods for confirming rupture of membranes (ROM) are obvious leakage of fluid from the cervix, positive nitrazine test, and ferning. Sonography may be used to confirm oligohydramnios, but is not confirmatory of ROM. Neither of these tests have been found to be 100% sensitive or specific. Despite the significant advances in technology, diagnosis of ROM still requires integration of symptomatology, physical examination, laboratory testing.

The present study was done to determine the sensitivity and specificity of the AmniSure rapid immunoassay for the detection of true PROM and PPROM.

SUBJECTS AND METHODS

The study was carried out in women presenting with ROM to the labor room of a referral rural medical institute in Central India after approval from Ethical Committee. A total of 200 consecutive pregnant women who had reported with complaints of watery vaginal discharge after 28 weeks gestation, were preterm, 68.5% (137/200) term. Statistical analysis of data collected in the electronic database using SPSS version (Amnisure International LLC, 30 JFK Street, 4th Floor, Cambridge, MA 02138, USA).

Results: AmniSure rapid immunoassay, rapid method for diagnosis of ROM, has 100% specificity, 99.44% sensitivity (one false negative due to meconium and immediate cesarean section).

Conclusion: In comparison to nitrazine, pooling, ferning, AmniSure has almost 100% sensitivity, specificity.

KEY WORDS: AmniSure, diagnosis, meconium, preterm, term rupture of membranes
An informed written consent was taken for each case. After a thorough history and clinical examination to exclude patients with vaginal bleeding, a blind vaginal swab was placed in the vagina for 1 min, and then dipped for 1 min in the solvent vial provided with the AmniSure test kit (AmniSure International LLC, Boston, MA, USA). The sample was then stored to be evaluated by the research assistant, who was blinded to the findings of the clinical examination. The AmniSure strip was dipped in the solvent for 5 min, and the result was observed and interpreted. The presence of two lines indicated a positive result, while a single line indicated a negative result. Sterile speculum examination was performed to observe pooling of liquor, collect specimens for the nitrazine and ferning tests. Sterile vaginal examination was then performed in term cases to determine cervical dilatation, effacement, and station of the presenting part. If all or at least, 2 out of the 3 traditional tests (i.e. pooling, ferning, and nitrazine) were positive, the membranes were considered to be clinically ruptured. However, if 2 out of 3 tests were negative, then a speculum examination was done with aseptic precautions, 30 min after the first, to allow the nitrazine, pooling, and ferning tests to be run again. Final test performance was calculated by comparing the results of the AmniSure test against final diagnosis at birth and the results of other tests performed during the evaluation.

An obstetric ultrasonography (USG) was performed for gestation, presentation, congenital anomalies, and amniotic fluid index (AFI). USG revealing oligoamnios further suggested ROM. The management protocol for confirmed PROM was followed. All the results were documented in the data sheets used for analysis. Statistical analysis of data collected the electronic database was done using SPSS version.

RESULTS

Of 200 women, two were younger than 20 years of age, 115 between 20 and 24 years, 62 between 25 and 29 years, 16 between 30 and 34 years and five were 35 years or above. A total of 120 women were primigravida, 47 second gravida, 20 third gravida, 9 fourth gravida, and 4 were fifth gravida. Twenty-four women had gestation between 28 and 32 weeks, 39 between 33 and 36 weeks (63 preterm), 119 between 37 and 40 weeks, and 18 between 40 and 42 weeks (137 term). Of 63 women with preterm gestation, 54 (85.72%) were diagnosed to be having ROM and 9 (14.28%) no ROM. Of the 137 women with term gestation, 127 (92.70%), had ROM, but 10 (7.29%) did not. Overall, 181 (90.5%) were diagnosed to have ROM and 19 (9.5%) no ROM at initial evaluation [Table 1].

In 19 cases, the AmniSure test was negative, in 180 cases, it was positive and in one case, the liquor had a lot of meconium, the woman had to undergo an emergency cesarean section, so second speculum examination (as per the protocol in cases of an initial negative result) could not be done for this case. This patient was deemed to have a false negative result by the AmniSure test. Ideally for false negative, repeat test should have been done. Pooling was positive in 177 cases, nitrazine test in 173 cases, and ferning in 178 cases. Hence, in comparison to AmniSure test which had 100% specificity and 99.44% sensitivity due to incomplete evaluation, nitrazine test had 95.58% sensitivity and 100% specificity. Pooling has 97.79% sensitivity and 100% specificity, whereas ferning has 98.34% sensitivity and 100% specificity.

At the time of admission, the majority of women complained of leaking for more than 24 h prior to their visit, and AFI was typically between 6 and 8 cm. Some patients reported leakage for <6 h prior to presentation, and the AFI was either <3 cm or between 3 and 5 cm [Table 2].

Of 181 women with confirmed ROM, 45 had a cesarean section, 39 of which were directly related to ROM, the remaining 6 were done for other reasons. Of 19 women with no ROM, 8 underwent cesarean section for various reasons. Overall 39 cesarean sections were done in women with term pregnancy and 14 in women with preterm gestation.

Overall, of the 181 women who had ROM, 1 had intrauterine death, 1 had stillbirth, 3 babies had neonatal death, and 20 (11.04%) babies were admitted to the neonatal intensive care unit (NICU). Of the 19 babies of women who had no ROM, 9 (47.36%) were admitted to the NICU. In total, there

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were six perinatal deaths (3.09%). The causes of deaths included small for gestational age, low birth weight, sepsis, severe birth asphyxia, respiratory distress and hydrops fetalis, the perinatal deaths possibly linked to ROM were five.

Overall of 200 cases in whom AmniSure test was performed, 180 were true positive, 19 were true negatives, 1 was a false negative, as repeat testing was not done. There was no false positive. Accordingly, the AmniSure test had 100% specificity and almost 100% sensitivity also. In one case in which the test was false negative, the liquor had a lot of meconium and on account of emergency cesarean section; a second AmniSure test could not be done, so ideally no comments can be made about only one false negative.

### DISCUSSION

Rupture of membrane is largely a clinical diagnosis. It is typically suggested by history of watery vaginal discharge on sterile speculum examination. The traditional diagnosis of ROM is by clinician’s ability to document three clinical signs on sterile speculum examination: (1) Visual pooling of clear fluid in posterior fornix of vagina or leakage of fluid from cervical os; (2) an alkaline pH of cervicovaginal discharge, demonstrated by nitrazine test; and/or (3) ferning of cervicovaginal discharge on drying. Moreover, sensitivity and specificity of the nitrazine test has been reported at 90-97% and 16-70%, respectively.[6,10] In the present study also, it was found that the nitrazine test had 95.58% sensitivity and 100% specificity, pooling has 97.79% sensitivity and 100% specificity, ferning has 98.34% sensitivity and 100% specificity. Further, the use of all three tests is not mandatory for diagnosis of ROM, when 2 of 3 are positive, clinical correlation is exercised.

Amnio-dye infusion is the only procedure that provides a 100% accurate result. After amniocentesis, a dye (Evans Blue or fluorescein) is injected into the amniotic cavity, and if a tampon placed in the vagina is stained blue following the procedure, ROM is confirmed unequivocally. However, this procedure is invasive, expensive, and is associated with risk to the pregnancy, including bleeding, infection, iatrogenic ROM, and possibly, loss of pregnancy (approximately 1 in 270).[11,12] Hence, this procedure is not used in day to day practice. Evidence of diminished amniotic fluid volume (by Leopold’s examination or ultrasound) alone cannot confirm the diagnosis,[3] but may help in the clinical setting. With the possible exception of direct visualization of amniotic fluid spurring from cervical os, all of these clinical signs have limitations in terms of diagnostic accuracy, cost, and technical ease. Moreover, such tests become progressively less accurate when more than 1 h has elapsed after membranes have ruptured or in certain nonobvious circumstances, where fluid may not be present in vagina for evaluation or what is present may be contaminated with urine, cervical mucus, vaginal discharge, blood, or meconium. Because of these difficult situations, multiple cytological, biochemical, colorimetric, and sonographic methods have been proposed for detection of ROM, however, no one test has been found to be completely accurate and diagnosis often requires an integration of historic factors, physical examination, and laboratory testing.

Many trials aimed at finding more accurate ways to detect ROM in nonobvious cases have evaluated biochemical markers including those based on the detection of fetal fibronectin,[13,14] alpha feto protein,[15] β-human chorionic gonadotropin in cervicovaginal secretions,[16] and insulin-like growth factor binding protein (IGFBP).[15,17] None of these markers, however, have proven to be reliable means to accurately diagnose true ROM. A recent study has revealed that IGFBP-1 is also an effective method with high sensitivity and specificity for the diagnosis of PROM.[18] To the contrary, investigators have demonstrated an excellent performance for test based on detection of placental alpha microglobulin-1 (PAMG-1), a rapid 5 min noninstrumented qualitative immunochromatographic test cleared by the FDA in 2004 (AmniSure ROM test, AmniSure International LLC, Boston, MA, USA). The test employs monoclonal antibodies, detects even a miniscule amount of PAMG-1, which is found in high concentrations in cervicovaginal secretions after ROM has occurred. AmniSure is believed to be highly accurate (over 99% sensitivity) and is easy to use.[19-26]

In the present study, 200 women who had presented with watery discharge, but did not have obvious membranes ruptured were evaluated for ROM by AmniSure test. Finally, the diagnosis of ROM was determined at birth. Overall, 200 cases available for analysis, the AmniSure test was
positive in 180 of these, 19 were true negatives, 1 was false negative and there was no false positive. Accordingly, the AmniSure test had 99.44% sensitivity, though with problem of loss of the second test and 100% specificity.

The study revealed excellent accuracy of the AmniSure test in the Indian population presenting with watery vaginal discharge. In comparison to nitrazine, pooling, and the ferning tests, the AmniSure test had better sensitivity and specificity.

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