

Nonintrusive Diagnosis of Premature Ruptured Amniotic Membranes Using a Novel Polymer

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ABSTRACT

This article describes the evaluation of the diagnostic efficacy of AL-SENSE panty-liner in detecting premature rupture of membranes (PROM). One hundred and three women attending the labor and delivery ward were enrolled into three groups: women presenting with a vaginal leak of fluid who had not yet been examined; women with overt PROM, and women with no fluid leak. The result of the AL-SENSE strip test was compared with the clinical diagnosis, which was based on direct visualization of the posterior vaginal fornix and cervix, crystallization, and nitrazine tests. AL-SENSE panty-liner test had a sensitivity of 100% and a specificity of 75% in detecting PROM, with an overall agreement of 82.35% between the AL-SENSE test result and the clinical diagnosis. AL-SENSE may be used as a reliable test to rule out PROM and as an effective device to diagnose PROM and differentiate it from urine leak and vulvovaginal candidiasis.

KEYWORDS: Premature rupture of membranes (PROM), diagnosis, chorioamnionitis, polymer, panty-liner, pH, nitrazine, ferning, AL-SENSE

Premature rupture of membranes (PROM) complicates 5 to 10% of pregnancies, and is associated with an increase in perinatal morbidity and mortality if not diagnosed in time. The optimal method for diagnosing PROM is controversial. Furthermore, the diagnosis of PROM is difficult when the fluid leak is slow, if there is spotting, and when the classic “gush of fluid” does not occur. In addition, many pregnant women experience urinary incontinence during the third trimester. Candida vaginitis or bacterial vaginosis are also possible causes of vaginal discharge that can mimic PROM.

Currently, when PROM is suspected, the following methods of evaluation are used: a speculum exami-

nation in an attempt to visualize a collection of fluid in the posterior fornix, mainly while the patient is asked to perform a Valsalva maneuver or cough, and the use of Litmus paper or nitrazine indicator to determine the pH of fluid obtained from the vaginal pool during speculum examinations.¹ Although normal vaginal pH is 3.5 to 4.5, amniotic fluid pH is above 6.0, which can be determined using Litmus paper. A false-negative nitrazine test can occur when there is only a tiny amount of fluid leak, such as in chronic membrane rupture or so-called high rupture of the membranes.

Microscopic techniques include the identification of fetal lanugo hair in the fluid sample, the

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demonstration of fetal fat particles (stained with Sudan as well as orange staining of the neutral lipid in desquamated fetal epithelial cells using the Nile blue sulfate stain), or the detection of fetal cells by various stains and techniques. Microscopic detection of a crystallization pattern (ferning test) is based on a phenomenon typical of amniotic fluid. However, cervical mucus might also cause a positive crystallization test.²

Recently introduced tests include analysis for α -fetoprotein, prolactin, fetal fibronectin, insulin-like growth factor binding protein-1, human placental lactogen, human chorionic gonadotropin, or placental α microglobulin-1 in the vagina. These markers are present in high concentrations in amniotic fluid but not in normal vaginal secretions.³ These tests require laboratory processing of the specimen, which has been collected using a swab taken from vaginal secretions. The tests are expensive and have a reasonably high negative-predictive value but a low positive-predictive value, rendering them appropriate for ruling out PROM but not for its confirmation.³ Ultrasound examination of the uterus, looking for a reduction in amniotic fluid volume, has also been advocated, but is obviously an indirect test for PROM.

An invasive technique, using an intra-amniotic injection of blue dye (indigo carmine) and checking for its appearance in the vagina or on perineal pads, is rarely performed.

Recently, a diagnostic panty-liner kit (AL-SENSE; CommonSense, Caesarea, Israel) has been developed. The test is especially appropriate for the detection of minute volumes of fluid because the patient wears the panty-liner for an hour before the evaluation is performed; hence it represents the fluid collected over a period of time. It contains a novel nitrazine (phenolate) ion polymer, which is capable of differentiating amniotic fluid from urine; the presence of urine in the tested fluid reverses the initial blue stain back to yellow. The reverse reaction is caused by detachment of conjugate-based nitrazine molecules by the urine ammonium ions while the strip dries. The higher the concentration of ammonium ions in the tested fluid, the more ions can attach to the ionized nitrazine molecules, reversing the blue color. No other positively charged ions can detach the phenolate ion while the strip dries. The polymer serves as a pH indicator that changes color upon contact with fluid of $\text{pH} > 5.2$.

This study evaluates the effectiveness of the AL-SENSE panty-liner in diagnosing PROM.

MATERIALS AND METHODS

One hundred eight pregnant women between the ages of 20.0 and 39.8 years (average age, 29.1; standard deviation, 4.45) were enrolled consecutively in three groups: study, negative control, and positive control

groups were enrolled. The study participant enrollment took place in the labor and delivery room of a university-affiliated hospital between April 14, 2002, and August 26, 2002. The inclusion criteria for subject participation in the study were more than 20 weeks of gestation arriving to Labor and Delivery suites; age between 18 and 45 years; and willingness to sign the informed consent form. The exclusion criteria were inability or unwillingness to cooperate with study procedures, or current participation in another clinical study.

The women were assigned to one of three groups: The study group comprised 34 women presenting with a vaginal leak of fluid, who had not yet been examined. The positive-control group comprised 42 women with overt spontaneous PROM (32 women) or artificial rupture of membranes (10 women). A negative-control group consisted of 27 women presenting for routine antepartum examination with no complaints of fluid leak. All women were selected throughout the study period. All provided written informed consent for study participation. The institutional review board of the participating hospital approved the study protocol.

THE PANTY-LINER

The AL-SENSE panty-liner consists of a polymer embedded in a test strip inserted into a panty-liner that changes color from yellow to blue-green upon contact with amniotic fluid. The AL-SENSE distinguishes between amniotic fluid and urine even if the urine has a pH similar to the amniotic fluid. This is due to the polymer matrix, which has a specific composition of ingredients that reverses the color change back to normal (yellow) by reacting to ammonia that is present in the urine within 10 minutes of drying in free air. The AL-SENSE underwent cytotoxicity, skin irritation, and sensitization tests and complies with the U.S. Pharmacopoeia Guidelines.

CLINICAL DIAGNOSIS

The diagnosis of amniotic fluid leakage was made (clinical diagnosis) by either finding obvious pooled amniotic fluid in the posterior fornix, or, in the absence of obvious fluid, detection of ferning or a positive nitrazine paper test. Every participant was given one amniotic fluid diagnostic pad (AL-SENSE) panty-liner upon arriving at the labor and delivery room. The pad was used for 1 hour in the reception area. Then, the woman was asked to remove the indicator strip from the pad, insert it into the plastic drying box, record the color change of the indicator strip, and mark if it changed color to blue or green (positive result). After 10 minutes of drying, a midwife or obstetrician and study coordinator (all were blinded to the patient-recorded color

change) checked the color of the indicator strip and recorded its final color (final result). The diagnosis of amniotic fluid leakage was made by a physician (other than the clinician who records the AL-SENSE results) who was blinded to other results.

In cases where the AL-SENSE pad showed a positive result but the clinical diagnosis did not suggest PROM, the possibility of bacterial vaginosis or trichomonas infection was assessed by microscopy.

Differences between the AL-SENSE final result reported by the clinician and clinical diagnosis in all cases were examined. Sensitivity, specificity, and overall agreement were calculated. Exact binomial 95% confidence intervals were calculated for all proportions reported. In addition, the κ statistic and 95% confidence interval were calculated to evaluate the inter-rater agreement between the study clinician evaluation and the study coordinator evaluation. This study was conducted as a feasibility study to estimate the accuracy of the panty-liner. Given that the purpose of the study was estimation, power analysis was not appropriate. A p value of 5% or less was considered statistically significant. The analysis was performed with SAS software, version 9 (SAS Institute, Cary, NC).

RESULTS

Of 108 women enrolled, five women were excluded from evaluation because of protocol violations (three did not complete the tests; one patient did not use the AL-SENSE according to instructions, and one patient had vaginal bleeding while using the panty-liner).

Gestational age and demographic data follow for the 103 valid cases: 92 (89.4%) of the participants were 36 weeks of gestation and above, six (5.8%) were between 26 and 35 weeks of gestation, and five (4.8%) were between 20 and 25 weeks of gestation. Most participants were Israeli (81%), salaried employees or homemakers (95%), and most of them had completed high school or an academic education (92%).

Table 1 compares the AL-SENSE panty-liner test readings to the clinical diagnosis in the 34 women of the study group who presented with suspected amniotic leak. Of the 34, in 10 a clinical diagnosis of PROM was

made. AL-SENSE correctly detected PROM in all of those cases. Twenty-four of the 34 women were clinically diagnosed as having intact membranes (no PROM). The AL-SENSE test was falsely positive in six of those 24 patients. Four of the six had bacterial vaginosis and in the other two women, clinical PROM developed a few hours after the initial negative clinical result. Thus, the AL-SENSE demonstrates a sensitivity of 100% (10 of 10) with 95% exact binomial confidence interval of 69.15 to 100% and a specificity of 75% (18 of 24) with 95% exact binomial confidence interval of 53.29 to 90.23%. The overall level of agreement between the AL-SENSE results and clinical diagnosis was found to be 82.35% (28 of 34), with 95% exact binomial confidence interval of 65.47 to 93.24%.

The positive-control group consisted of 42 women who had overt spontaneous PROM (32 women) or had undergone amniotomy (10 women). AL-SENSE correctly diagnosed all of those cases (data not tabulated).

In the negative-control group, 25 of the 27 women indeed had unruptured membranes. AL-SENSE correctly detected 23 of the 25 cases. In the other two women, AL-SENSE gave a false-positive result, suggesting PROM. Two of the 27 women had ruptured membranes, The AL-SENSE correctly detect both of them (data not tabulated).

The κ value was calculated between the study clinician evaluation and the study coordinator evaluation for 103 subjects; the κ statistic was 0.8137 with 95% confidence interval of 0.6829 to 0.9446. This indicates a moderate to high level of agreement between the two assessments.

DISCUSSION

The correct diagnosis of PROM is crucial because prompt, appropriate management can obviate serious maternal and neonatal complications.⁴ If possible, during evaluation of suspected preterm PROM, vaginal and cervical examinations should be avoided because of the risk of ascending infection. Lewis et al⁵ also found that digital vaginal examinations performed on patients with preterm PROM significantly shortened the latency period at all gestational ages (2.1 ± 4.0 versus 11.3 ± 13.4 days; $p < 0.0001$). Alexander et al⁶ reported that performance of one or two digital cervical examinations during expectant management of preterm PROM (24 to 32 weeks of gestation) was associated with a median of 2 days shorter latency (3 versus 5 days; $p < 0.009$). The possible increase in the infection rate of the use of a vaginal swab, as is required by most of the newer diagnostic test for PROM, has not yet been evaluated, but a completely noninvasive test might conceivably lead to a lower rate of ascending infection. Therefore, a diagnostic technique that avoids intravaginal examination or speculum insertion is desirable. The AL-SENSE

Table 1 Study Group: Patients Presenting with Suspected PROM

AL-SENSE Test Result	Clinical Diagnosis		Total
	Positive	Negative	
Positive	10	6	16
Negative	0	18	18
Total	10	24	34

The data in the table were used to calculate false-positive and false-negative rates for the study group (data presented in the text). PROM, premature rupture of membranes.

panty-liner test was shown to be a sensitive tool for excluding PROM and differentiating amniotic fluid leaks from urine incontinence. In our study, in which the prevalence of PROM was 52.43%, the panty-liner test had a 100% negative predictive value. It can therefore be used with confidence to rule out suspected PROM. The false-positive cases were limited to a few women with bacterial vaginosis infection or in patients in whom PROM occurred a few hours after the so-called false-positive detection (Table 1).

When vaginal fluids with pH > 5.2 contact the indicator strip, the color changes from yellow to green or blue. In women with bacterial vaginosis and/or trichomoniasis, where the vaginal discharge may exceed pH 5.0, the AL-SENSE indicator strip also resulted in positive results. On the other hand, in women with candida vaginitis, the vaginal discharge has a normal pH (pH ≤ 4.5), thus the AL-SENSE indicator strip remained negative (submitted for publication).

It should be noted that the study was performed on a limited sample size (34 participants), and the statistical significance is limited.

The efficacy of the AL-SENSE may be attributed to the continuous collection of the amniotic fluid rather than spot checking, as is the case with other tests. The panty-liner reflects the condition over an hour or a few hours prior to examination. Therefore, even if the fluid escapes periodically in small amounts and not continuously (such as in a so-called high leak), the AL-SENSE can detect it.

An additional advantage of the AL-SENSE panty-liner, in contrast to traditional tests, is the option of self-application by the patient. This is useful for a pregnant patient who suffers from recurrent vulvovaginal candidiasis, to ascertain whether a discharge represents amniotic fluid or another episode of candida vaginitis. Similarly, patients with urinary incontinence caused or enhanced by pregnancy may obtain reassurance that PROM has not occurred by testing the discharge with the AL-SENSE and finding that the discharge is, in fact, urine. The AL-SENSE may also have important medicolegal and economic implications in the manage-

ment of inpatients. The option of self-application enables physicians to discharge patients initially admitted to the hospital with suspicion of PROM, to instruct them to continue monitoring for possible PROM using the AL-SENSE, and to return for further observation once the panty-liner indicates a change in color to blue-green. This approach can prevent prolonged unnecessary hospitalization and reduce patient anxiety.

In conclusion, the present study demonstrates that the new panty-liner kit, AL-SENSE, is a highly sensitive, noninvasive method for use in ruling out the possibility of PROM. It is also capable of differentiating PROM from urinary incontinence or vulvovaginal candidiasis, thereby avoiding misdiagnosis, unnecessary treatment, and interventions, which reduces patient anxiety and lowers costs.

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