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Original Research

Evaluation of simple and low-cost diagnostic tests for premature rupture of membranes

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Summary

Purpose of Investigation: To determine the accuracy from three diagnostic methods for premature rupture of membranes. *Materials and Methods:* Samples from 100 pregnant women were divided into two groups: intact and ruptured membranes. Samples of 2-5 ml were collected from the vaginal content and the exams were performed, analysing accuracy measures. *Results:* The pH demonstrated sensitivity of 100.0%, specificity of 96.0%, positive predictive value (PPV) of 96.2%, negative predictive value (NPV) of 100.0%, and accuracy of 96.0%. Iannetta test demonstrated sensitivity of 86.0%, specificity of 92.0%, PPV of 91.5%, NPV of 86.8%, and accuracy of 79.1%. Crystallization demonstrated sensitivity of 90.0%, specificity of 98.0%, PPV of 10.00%, NPV of 90.7% and accuracy of 88.2%. *Conclusion:* The most specific test with higher PPV was crystallisation, alone or in combination with others (pH and Iannetta test). The most sensitive test with higher NPV and accuracy was pH, alone or in association with others (Iannetta test and crystallization).

Key words: Premature rupture of membranes; Diagnostics; Amniotic fluid.

Introduction

Premature rupture of membranes has been the most commonly used term to determine the rupture of the amnion and chorion at any time before labor begins, during the second half of pregnancy [1-3].

Premature rupture of membranes is one of the most common problems in perinatal medicine. It is the obstetric disease most associated with preterm birth worldwide, becoming an important cause of maternal and fetal morbidity and mortality [1, 2]. Its incidence varies widely between 2.7 and 17.0% [4, 5], but many authors refer it to be around 10.0% of all pregnancies [1, 3-7]. The management of a patient with such a condition in term or non-term pregnancies remains a major problem. Its diagnosis may be uncertain and the failure to confirm it may cause great maternal and fetal risks [7]. Also, a false diagnosis may lead inappropriately to the interruption of a normal and viable pregnancy [7]. Therefore, all precautions should be taken to diagnose this condition as quickly and accurately as possible [7-9]. Anamnesis and physical exam alone are, in most cases, sufficient to confirm the membranes' state due to the amniotic fluid detected by a vaginal speculum [4, 10]. However, the amniotic fluid may not be present in the vagina. If present, may be contaminated by urine, cervical mucus, vaginal secretions, blood, meconium, infections or other substances. Due to these difficulties, cytological, biochemical, echographic, and staining methods have been developed for the detection of premature rupture of membranes [7, 11, 12]. Despite significant advances in technology, no test has demonstrated complete accuracy and the diagnosis, even today, requires the integration of compatible story, physical examination and laboratory tests [13]. Some of theses tests, such as pH determination, the Iannetta test, and crystallization tests are used to detect the presence or not of the amniotic fluid [12-15]. The alkalinity of the amniotic fluid allows its detection in the vaginal cavity using a tape of pH [7].

The Iannetta test is based on the staining acquired by vaginal secretion after drying by heating on a glass slide for one minute [10]. The crystallization test allows the identification of a fern pattern of the vaginal secretion visible in an optical microscope [10].

These methods have advantages when compared to others, once they involve simple and non-invasive procedures, low cost, minimal risks, and no need of specialized personnel and equipment, and may be performed at the patient's bedside with immediate results [16].

The objective of this study was to describe the accuracy from three diagnostic methods for premature rupture of membranes (pH, Iannetta test, and crystallization), alone and in association.

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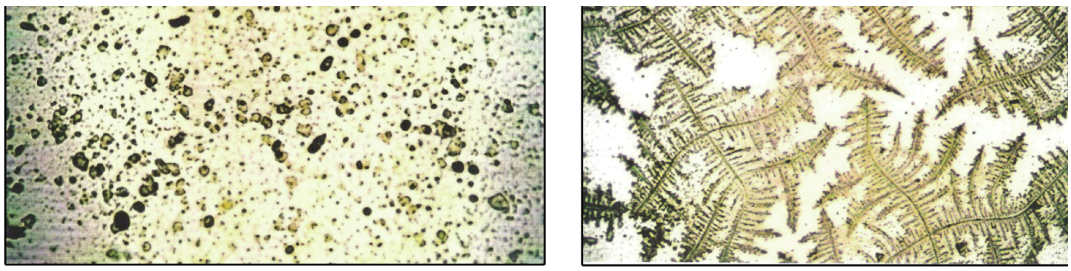

Figure 1. — Pattern of the crystallization of cervical mucus (left) and amniotic fluid (right) after spontaneous stainning at a ×40 magnification with optical microscopy.

Materials and Methods

An accurate study was carried out, involving 100 pregnant women randomly selected among those who were admitted to the maternity of a public hospital, in the city of São José, Brazil. Pregnant women between 37 and 41 weeks and six days were included. Patients presenting vaginal bleeding, leukorrhea, pregnancy-induced hypertension, gestational diabetes, and fetal death were excluded. They were divided into two groups: a) intact membranes (IM) group: those with no story of vaginal losses and no amniotic fluid from the external cervical orifice, even after Tarnier manouver [9]; b) ruptured membranes (RM) group: those patients diagnosed with spontaneous rupture of membranes confirmed by physical examination, and those where amniotomy was performed during labor. Sociodemographic data was obtained from the medical records. During a speculum examination, 2-5 ml of fluid were aspirated from the vaginal posterior fornix. Samples were tested for pH, Iannetta test, and crystallization.

To determine pH, an indicator tape was used by placing a drop of the fluid on it and observing the specific staining for the pH range. Values greater than or equal to seven were considered positive for amniotic fluid [7, 16].

For Iannetta test, a drop of the sample was placed on a glass slide and heated with a flame from a 10-cm distance for one minute. The sample was considered positive for amniotic fluid when the material was dried in White, and representing cervical mucus when dried in brown [12].

To perform the crystallization test, a drop of the sample was placed on a glass slide and spontaneously dried for ten minutes and then observed in an optical microscope. It was considered positive to amniotic fluid when the pattern of crystals as a "fern" was present [9] (Figure 1).

Statistical analysis was performed by the SPSS version 18. The sociodemographic characteristics of the participants were described in absolute and relative frequency. Sensitivity (S), specificity (E), positive predictive value (PPV), negative predictive value (NPV), and accuracy (A) of these tests alone and with associations in series and in parallel were analyzed.

The association of tests raises the quality of the diagnosis while decreasing the number of incorrect results. In the case of the parallel test, if one of the tests is positive, the test set is equally positive, therefore it is most useful in emergencies, when a quick approach is needed. The series test is considered positive only if all the individual tests are positive; in this case, the tests are applied consecutively, and the following test is applied only if the previous test is positive, while reducing costs.

This study followed the Helsinki recommendations and was approved by the Research Ethics Committee under CAAE 78646317.8.0000.5369. An informed consent was also obtained from all patients.

Table 1. — *Results of sensitivity and specificity of the diagnostic tests, isolated, and with associations.*

Diagnostic tests (isolated)	Sensitivity		Specificity	
pH	100.0%		96.0%	
Iannetta test	86.0%		92.0%	
Crystallization	90.0%		98.0%	
Diagnostic tests (associations)	Parallel	Series	Parallel	Series
pH and Iannetta test	86.0%	100.0%	99.7%	88.3%
pH and crystallization	90.0%	100.0%	99.9%	94.1%
Iannetta test and crystallization	77.4%	98.6%	99.8%	90.2%
pH and Iannetta test and crystallization	59.9%	100.0%	99.9%	74.9%

Table 2. — *Results of positive predictive values, negative predictive values, and accuracies of the diagnostic tests, isolated and with associations.*

Diagnostic tests (isolated)	PPV	NPV	Accuracy	
pH	96.2%	100.0%	96.0%	
Iannetta	91.5%	86.8%	79.1%	
Crystallization	100.0%	90.7%	88.2%	
Diagnostic tests (associations)			Parallel	Series
pH and Iannetta test			85.7%	88.3%
pH and crystallization			89.9%	94.1%
Iannetta and crystallization			77.3%	88.9%
pH and Iannetta test and crystallization			59.9%	74.9%

PPV = positive predictive value; NPV = negative predictive value.

Results

Samples were collected from a total of 100 women divided equally between the two groups. The age of patients ranged from 15 to 39 years, with mean of 29 ± 3.4 years. The majority of women were Caucasian (78%). Gestational age ranged from 37 weeks and three days to 41 weeks and four days. Regarding the pH test in the RM group, all samples were positive for the presence of amniotic fluid (pH 9-10). In the IM group, 48 (96.0%) samples were negative (pH 4-6) and two (4.0%) samples were positive for amniotic fluid (pH 8-9). Regarding the Iannetta test, the RM

group showed 43 (86.0%) positive samples and seven (14.0%) negative samples. In the IM group, 46 (92.0%) samples dried in brown and four (8.0%) in white. Regarding crystallization, 45 (90.0%) samples of the RM group demonstrated a positive pattern and five (10.00%) samples were considered negative. In the IM group, 49 (98.0%) were negative, however the positive pattern was found in one (2.0%) sample. All accuracy markers were above 70.0%, including the isolated and the associated analysis, including specificities, sensitivities, positive and negative predictive values, and accuracies. Greater specificity was demonstrated in parallel, while in series associations greater sensitivity and accuracy were found (Tables 1 and 2).

Discussion

High standard results were found, but it is important to note that the interpretation of the techniques used may consider some external factors in daily clinic (material changes, execution faults, and/or contaminants), and even if taking all precautions to avoid them, it might cause some false positive or negative results. Some results of this study were different from those obtained by other authors [7, 10, 17-19]. However all of them accept that the interference factors are the most important issue to determine the accuracy of a diagnostic test in premature rupture of membranes, and the false positive or negative results should be carefully considered and interpreted. The pH test was first described almost one hundred years ago [17]. In pregnant women, vaginal content is usually acidic, with a pH between 4.5 and 6.0, while the pH of the amniotic fluid is above 7.0. According to this test, the values found a specificity (96.0%) and sensitivity (100.0%) which corroborated with the results cited by other authors [2, 13]. This also occurred with the positive and negative predictive values (96.6% and 100.0%, respectively) [2, 7, 13, 19] and its accuracy (96.0%) [7, 13, 17, 18, 20].

When the vagina is contaminated by exogenous substances, such as semen, urine, alkaline soap, antiseptics, blood or meconium, and even a vaginitis, the pH values can change [7, 13, 17, 18, 20]. In theses situations, the pH test becomes less useful to diagnose the premature rupture of membranes. These situations could hypothetically explain the two false-positive results found in the present study.

According to Iannetta [12], the test proposed by him has a 100.0% accuracy. However, in this study, the results differed from those reported by the author. The white color that appears after heating the slade with the amniotic fluid is caused by the presence of electrolytes [12], and the brown coloration acquired by cervical mucus appears because of some proteins' carbonization [12, 21].

Among the seven samples of the RM group which dried in brown, four were contaminated with meconium, explaining part of the false-negative results. However, this factor presents no clinical reliance, because the presence of meconium in the vaginal content is an evidence of ruptured membranes. Another valuable test for the diagnosis of premature rupture of membranes is the crystallization pattern of the amniotic fluid, witch was first reported 50 years ago [13]. The pattern of the vaginal secretions does not exhibit this crystallization during pregnancy [7]. It occurs due to the higher concentration of proteins, carbohydrates, and electrolytes in the amniotic fluid [9]. However, this test can also present false results, because there are substances capable of producing arboriform patterns of crystallization, such as antiseptics, semen, and cervical mucus [13, 17-19]. Five false-negative results could be explained by the fact that two were contaminated with blood and the other three belonged to patients with latency time greater than four hours from the beginning of the fluid loss to the sample collection. In this research, specificity found for this test (98.0%) was exactly the same found in another study [13], but sensitivity (90.0%) was lower than those reported by other authors [13,17]. Positive (100.0%) and negative (90.7%) predictive values were comparable to other researches as well as accuracy (88.2%) [7, 10, 13, 17, 19, 20].

To improve sensitivity, specificity, and consequently accuracy of the diagnosis of premature rupture of membranes associations between two or more tests can be performed. These associations can occur in series to increase the sensitivity of the tests, improving the probability of excluding a rupture of the membranes, if all results are negative [22]. All associations in series with the pH test had the highest sensitivity (pH and Iannetta test, pH and crystallization, pH and Iannetta test and crystallization). The association in parallel increases the specificity of the tests, while improving the confirmation of rupture of membranes, if all tests have positive results [22]. Therefore, the association in series of the three tests presented the highest specificity (pH and Iannetta test and crystallization).

Conclusion

It is concluded that despite the difficulty to provide a clinical diagnosis of the premature rupture of membranes in some occasions, simple tests with high accuracy can be performed, isolated or in association, completing the diagnostic investigation while improving maternal and fetal outcomes.

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