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Research Article

AmnioQuick® Duo+ for diagnosis of premature fetal membranes rupture

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ABSTRACT

Background: Failure to identify women with premature fetal membranes rupture associated with infectious morbidities. Evaluation of the accuracy of AmnioQuick® Duo+ in diagnosing premature fetal membranes rupture compared to conventional diagnostic tests was the aim of this study.
Methods: 220 pregnant women ≥37 and <39 weeks` gestation studied and classified into two groups; study group (premature fetal membranes rupture) and control group (no premature fetal membranes rupture). Participants examined by trans-abdominal ultrasound (TAS) and vaginal speculum to visualize amnion leaking and for collection of samples for fern, nitrazine and AmnioQuick® Duo+ tests on admission. A final diagnosis whether the studied women had PROM or not at the initial presentation made after delivery.
Results: Sensitivity and specificity of the AmnioQuick® Duo+ to diagnose PROM was 93.6% and 86.4%; respectively compared with 72.7% and 80.9%; respectively for fern test and 76.4% and 83.6%; respectively for nitrazine test. Positive predictive value, negative predictive value and accuracy of AmnioQuick® Duo+ to detect PROM were 87.3%, 93.1% and 90%; respectively compared with 79.2%, 74.8% and 76.8%; respectively for fern test and 82.4%, 77.97% and 80%; respectively for nitrazine test. AmnioQuick® Duo+ test had higher accuracy to detect premature fetal membranes rupture compared to conventional diagnostic tests.
Conclusions: AmnioQuick® Duo+ is accurate bedside immunoassay test, better than the individual conventional diagnostic tests and can used as complementary test to improve the management of women with women premature fetal membranes rupture.

Keywords: AmnioQuick® Duo+, Premature fetal membranes rupture

INTRODUCTION

Premature fetal membranes rupture is rupture of the fetal membranes before the onset of uterine contractions, while rupture of fetal membranes before 37 weeks` gestation is defined as preterm premature fetal membranes rupture.[1,2] Premature fetal membranes rupture occurs in 2 -18% of all pregnancies, while, preterm premature fetal membranes rupture occurs in 0.7-4%.[3]

Management of ruptured fetal membranes (ROM) should be conservative if ROM occurs before 37 weeks` gestation, while, labor should induced if ROM occurs at term.[4]

Failure to identify women with premature fetal membranes rupture associated with infectious morbidities.[5-7]

ROM is a common problem in obstetrics due to absence of non-invasive diagnostic standards.[7] Absence of a non-

invasive diagnostic standards for diagnosis of ROM leads to appearance of biological tests based on alternative markers that highly present in amnion.[4]

These markers include; human chorionic gonadotropin, alpha-fetoprotein (AFP), fetal fibronectin, Placental alpha microglobulin-1 and insulin-like growth factor binding protein-1 (IGFBP-1).[7-18]

Recently, a new, rapid immunoassay test AmnioQuick® Duo+ test can detect IGFBP-1/ AFP in the amnion.[4] This study designed to evaluate the accuracy of IGFBP-1/ AFP (AmnioQuick® Duo+) test to detect premature fetal membranes rupture compared to conventional diagnostic tests.

METHODS

This comparative study conducted from July 2013 until July 2014 in Ahmadi Hospital, Kuwait. Two hundred and twenty participants' ≥37 and <39 weeks' gestation admitted for induction of labour studied after consent and approval of the study by ethical committee.

Participants' classified into two groups; study group (110 women with premature fetal membranes rupture) and control group (110 women without premature fetal membranes rupture) admitted for labour induction due to intrauterine growth retardation or diabetes or hypertensive disorders.

Women with ROM beyond 39 weeks' gestation or women with prolonged premature fetal membranes rupture (>12 hours) or twins pregnancy or non-reassured fetal tracing or ante-partum hemorrhage or preterm labour or infection of fetal membranes excluded from this study.

The premature fetal membranes rupture diagnosed by history of gush of amnion, positive fern and nitrazine tests, confirmed by amnion leaking from the cervix and AFI ≤5 cm by trans-abdominal ultrasound.[7,11]

Gestational age of the studied women calculated accurately from last menstrual period and early ultrasound scan done before 20 weeks.

Examination of studied women followed by trans-abdominal ultrasound and vaginal speculum examination to visualize amnion leaking and for collection of samples for fern, nitrazine and AmnioQuick® Duo+ tests on admission.

In addition, maternal fever and maternal leucocytosis, C-reactive proteins evaluated to exclude chorio-amnionitis.[19,20]

Studied women examined with proper illumination in Lithotomy position for samples collection using vaginal speculum.

Samples collected from vaginal fornix using 4 swabs. The first nitrazine yellow swab inserted for 15 seconds in the posterior vaginal fornix and then the colour of the swab checked.

The sample of the fluid collected by the second swab spreaded on a glass slide and examined by low power microscope (fern test).

Fourth Nylon swab supplied in the AmnioQuick® Duo+ (Biosynex, Strasbourg, France) kit package inserted in the posterior vaginal fornix for 1 minute and the IGFBP-1/AFP (AmnioQuick® Duo+) done according to instructions.

The test considered positive when both C and B lines present or when both A and C lines present. The test considered negative when both A and B lines absent. Test considered invalid when no visible purple band at line C.[4,21] (Figure 1 and Table 1).

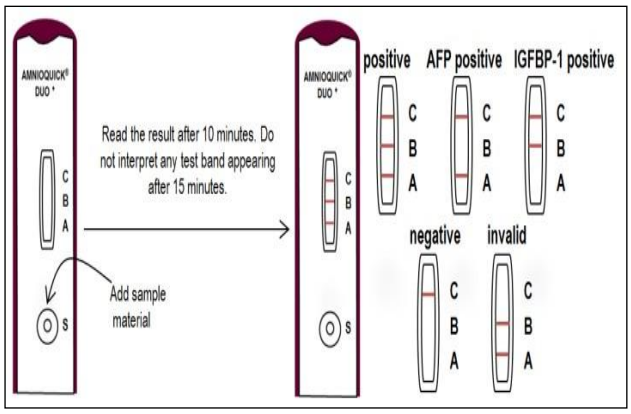


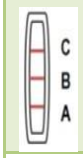
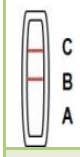
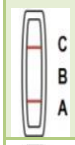
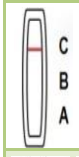
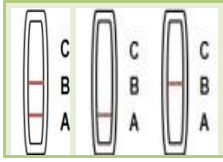
Figure 1: AmniQuick® Duo+ test results.

A final diagnosis of whether the studied women had premature fetal membranes rupture or not at the initial presentation made after delivery by blinded investigator to the AmnioQuick® Duo+ results. After delivery, the recorded information of admission and delivery analysed to compare AmnioQuick® Duo+ (IGFBP-1/AFP) to conventional diagnostic tests for diagnosis of premature fetal membranes rupture.

Sample size and statistics

Data of previous studies and G* Power software used for calculation of the sample size (*Heinrich Heine Universität; Düsseldorf; Germany).[5,7]

Table 1: Interpretation of AmniQuick® Duo+ test results.

Results of AmniQuick® Duo+ Test	Interpretation of the results	Conclusion
 IGFBP-1 and AFP Positive	Presence of 3 distinct purple lines: A control line appears at the level of the C zone and two purple lines (even of weak intensity) appear at the level of A and B zones.	Positive ROM
 IGFBP-1 positive and AFP Negative	Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line (even of weak intensity) appears at the level of B zone.	Positive ROM
 IGFBP-1 negative and AFP positive	Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line (even of weak intensity) appears at the level of A zone.	Positive ROM at GA ≥ 37 weeks' gestation
 Negative	Only one purple line appears at the level of the control line (C). No line appears at the level of the A and B zones	Negative
 Invalid	No visible purple band at the level of control line C (whatever A and B band apparition). Results from a test with no control line must discarded.	Invalid test

Data analysed using SPSS. Mean and standard deviation (±SD) used to present numerical variables, while number (n) and percentage (%) used to present categorical variables. Chi-square (X^2) used for analysis of qualitative data and t test for quantitative data. Accuracy of used tests was also calculated.

RESULTS

No statistical difference between study (PROM) and control group (no PROM) as regards; mean maternal age and gestational age.

AmnioQuick® Duo+ test was true positive (TP) in 103 women (93.6% sensitivity) and false negative (FN) in 7 women (6.4%) of the study group (PROM), while it was true negative (TN) in 95 women (86.4% specificity) and false positive (FP) in 15 women (13.6%) of the control group (no PROM).

Fern test was TP in 80 women (72.7% sensitivity) and FN in 30 women (27.3%) of the study group, while it was TN in 89 women (80.9% specificity) and FP in 21 women (19.1%) of the control group.

Nitrazine test was TP in 84 women (76.4% sensitivity) and FN in 26 women (23.6%) of the study group, while it was TN in 92 women (83.6% specificity) and FP in 18 women (16.4%) of the control group (Table 2).

Table 2: Results of AmnioQuick® Duo+, fern and nitrazine tests in studied groups.

Variables	Study group (PROM) Number 110		Control group (no PROM) Number 110	
	True positive cases (TP) No (%)	False negative cases (FN) No (%)	False positive cases (FP) No (%)	True negative cases (TN) No (%)
AmnioQuick® Duo test	103 (93.6%)	7 (6.4%)	15 (13.6%)	95 (86.4%)
Fern test	80 (72.7%)	30 (27.3%)	21 (19.1%)	89 (80.9%)
Nitrazine test	84 (76.4%)	26 (23.6%)	18 (16.4%)	92 (83.6%)

FN: False negative, FP: False positive, TN: True negative, TP: True positive, PROM: Premature rupture of fetal membranes

Sensitivity and specificity of AmnioQuick® Duo+ test to detect premature fetal membranes was 93.6% and 86.4%; respectively compared with 72.7% and 80.9%; respectively for fern test and 76.4% and 83.6%; respectively for nitrazine test.

PPV, NPV and accuracy of AmnioQuick® Duo+ test to detect premature fetal membranes were 87.3%, 93.1% and 90%; respectively compared with 79.2%, 74.8% and

76.8%; respectively for fern test and 82.4%, 77.97% and 80%; respectively for nitrazine test (Table 3).

Although, AmnioQuick® Duo+ test had higher sensitivity, specificity, predictive values and accuracy to detect PROM compared to conventional diagnostic tests (fern and nitrazine), this difference was statistically insignificant (Table 4).

Table 3: Accuracy of AmnioQuick® Duo+, fern and nitrazine tests to detect PROM in studied groups.

Variables	AmnioQuick Duo+ test	Fern test	Nitrazine test
Sensitivity=TP/(TP+FN) X 100	103/(103+7) X100 = 93.6%	80/(80+30) X100 =72.7%	84/(84+26) X100 = 76.4%
Specificity=TN/(TN+FP) X 100	95/(95+15) X 100 = 86.4%	89/(89+21) X100 = 80.9%	92/(92+18) X 100 = 83.6%
PPV=TP/(TP+FP) X 100	103/(103+15) X100 = 87.3%	80/(80+21) X 100 = 79.2%	84/(84 + 18) X 100 = 82.4%
NPV=TN/(TN+ FN) X 100	95/(95+7) X 100 = 93.1%	89/(89+30) X 100 = 74.8%	92/(92+26) X 100 = 77.97%
Accuracy=TP+TN/(TP+TN+FP +FN) X 100	103+95/(103+95+15+7) X 100 = 90%	80+89/(80+89+21+30)X 100 = 76.8%	84+92/(84+92+18+ 26) X 100 = 80%

FN: False negative, FP: False positive, NPV: Negative predictive value, PPV: Positive predictive value, TN: True negative, TP: True positive, PROM: Premature rupture of fetal membranes

Table 4: Accuracy of AmnioQuick® Duo+ to detect PROM compared to fern and nitrazine tests.

Variables	AmnioQuick Duo+ test	Fern test	Nitrazine test	p Value, Significance
Sensitivity	93.6%	72.7%	76.4%	*p = 0.22 (>0.05), NS **p = 0.32 (>0.05), NS
Specificity	86.4%	80.9%	83.6%	*p = 0.74 (>0.05), NS **p = 0.87 (>0.05), NS
Positive predictive value (PPV)	87.3%	79.2%	82.4%	*p = 0.63 (>0.05), NS **p = 0.77 (>0.05), NS
Negative predictive value (NPV)	93.1%	74.8%	77.97%	*p = 0.28 (>0.05), NS **p = 0.38 (>0.05), NS
Accuracy	90%	76.8%	80%	*p = 0.44 (> 0.05), NS **p = 0.56 (>0.05), NS

*P-value when AmnioQuick® Duo+ test compared to fern test, **P-value when AmnioQuick® Duo+ test compared to nitrazine test, Chi-square (X^2) used for statistical analysis, NS: Non-significant, PROM: Premature rupture of fetal membranes

DISCUSSION

Failure to identify women with premature fetal membranes is associated with infectious morbidities.[1,2,5-7] False positive results are high with conventional diagnostic tests (fern and nitrazine) used to diagnose premature fetal membranes rupture.[7]

AmnioQuick® Duo+ can detect amniotic fluid in a sample of vaginal secretions through identification of IGFBP-1 and AFP whose concentration in amnion is very high.[4,21]

IGFBP-1 level in the amnion is 100-1000 times higher than in the serum. Threshold for IGFBP-1 detection using AmnioQuick® Duo+ is 10 ng/ml.[4]

Because the threshold for IGFBP-1 detection using AmnioQuick® Duo+ is 10 ng/ml, the PPV of AmnioQuick® Duo+ for IGFBP-1 is therefore very high.[4,21]

The concentration of AFP is fluctuating during pregnancy, it significantly decreases during the 3rd trimester of pregnancy and because the threshold of AmnioQuick® Duo+ for detection of AFP is 5 ng/ml, the PPV of AmnioQuick® Duo+ for AFP is very high beyond 37 weeks` gestational age.[4,21]

Women with ante-partum hemorrhage excluded from this study because presence of blood in the collected samples can lead to false positive results of AmnioQuick® Duo+ test.

In addition, because, prolonged premature fetal membranes rupture (>12 hours) increases liability of IGFBP-1 degradation by vaginal proteases and AFP

concentration decreases in amniotic fluid beyond 39 weeks` gestation, women with prolonged PROM and women with premature fetal membranes rupture beyond 39 weeks` gestation excluded from this study.[4,12,21]

In this study, the AmnioQuick® Duo+ test had 93.6% sensitivity, 86.4% specificity, 87.3% PPV, 93.1% NPV and 90% accuracy.

Ruanphoo et al, concluded that; AmnioQuick® Duo+ had 94.1% sensitivity, 87.5% specificity, 97.5% PPV, 73.7% NPV and 93% accuracy.[4]

In addition, Thomasino et al, evaluated the performance of combined monoclonal/polyclonal immunoassay test using placental protein-12 (PP12) /AFP (ROM Plus) to diagnose ROM and they found that the combined PP12/AFP immunoassay had 99% sensitivity, 91% specificity, 95% PPV and 99% NPV.[22]

In this study, AmnioQuick® Duo+ test had higher sensitivity and specificity in detection of PROM (93.6% and 86.4%; respectively) than fern test (72.7% and 80.9%; respectively) and nitrazine test (76.4% and 83.6%; respectively). In addition, AmnioQuick® Duo+ test had higher PPV, NPV and accuracy in detection of PROM (87.3%, 93.1% and 90%; respectively) compared to fern test (79.2%, 74.8% and 76.8%; respectively) and nitrazine test (82.4%, 77.97% and 80%; respectively).

Ruanphoo et al concluded that; the AmnioQuick® Duo+ test had higher sensitivity than the conventional methods in diagnosing ROM, because the AmnioQuick® Duo+ test detect two markers in the amniotic fluid at very low threshold (IGFBP-1 at 10 ng/ml and AFP at 5 ng/ml).[4]

Thomasino and colleagues found that combined PP12/AFP (ROM Plus) immunoassay to diagnose ROM had 99% sensitivity, 91% specificity, 95% PPV and 99% NPV compared to 99% sensitivity, 72% specificity, 80% PPV and 99% NPV for fern test and 93% sensitivity, 83% specificity, 90% PPV and 88% NPV for nitrazine test.[22]

Thomasino et al, concluded that the combined immunoassay test using PP12/AFP for detection of ROM was better than the individual components of conventional tests (fern and nitrazine).[22]

In this study, AmnioQuick® Duo+ test had higher sensitivity, specificity, predictive values and accuracy to detect premature fetal membranes rupture compared to conventional diagnostic tests, this difference was statistically insignificant.

Ruanphoo et al, in their study mentioned the accuracy of IGFBP-1/AFP (AmnioQuick® Duo+) and standard methods in diagnosing ROM, without statistical analysis or comparison between used diagnostic methods. Also, Thomasino et al, in their study mentioned the accuracy of PP12/AFP (ROM Plus) immunoassay test, conventional clinical evaluation, fern and nitrazine tests to diagnose ROM without statistical analysis or comparison between used diagnostic methods.[4,22]

The strength of this study is coming from participants' selection, proper sample size and statistical analysis, in addition to comparative (case control study) nature of the study.

Because AmnioQuick® Duo+ test is new, immunoassay test detect two biological markers present in the amniotic fluid, little available data and studies about AmnioQuick® Duo+ test was the only limitation faced during this study

More comparative studies needed to compare the accuracy of AmnioQuick® Duo+ with other immunoassay tests like PAMG-1 in diagnosis of PROM.

CONCLUSIONS

This study concluded that IGFBP-1/AFP (AmnioQuick® Duo+) test is accurate bedside immunoassay test, better than the individual conventional diagnostic tests and can used as complementary test to improve the management of women with women premature fetal membranes rupture.

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