Conclusion

We can conclude that the use of AmnioQuick dipstick test for detection of IGFBR-1 in cervico-vaginal secretions to diagnose premature rupture of membranes is a simple, rapid, non-invasive and reliable method with a very high sensitivity and reasonable specificity. Larger studies involving mainly women with suspected membrane rupture can test the reliability of AmnioQuick test to focus light on its relatively high false positive rate which is considered an important limitation of its use for screening of PROM. Higher specificity is needed to avoid unnecessary obstetric measures that may harm the mother or her fetus.

PATIENTS AND METHODS

This prospective case-control study was carried out at Ain Shams University Maternity Hospital during the period between June 2011 to January 2012.

Sample size calculation:

The required Sample size has been calculated using the Power Analysis and Sample Size software v. 08.0.9 (PASS©, NCSS LLC, Kaysville, UT, USA).

The primary outcome measure has been the proportion of patients who will be correctly diagnosed by examination of amniotic fluid for presence or absence of (IGFBP-1).

It has been estimated that a sample size of 21 patients in each of the three study groups would achieve an 80% power to detect an effect size of 0.4 using a two-degrees-of-freedom chi-square test with a significance level (α-error) of 0.05.

The study included 63 pregnant women between 24-36 weeks of gestation divided into three groups:

Group I: (Confirmed PROM)

- This included 21 preterm pregnant women between 24-36 weeks of gestation who had confirmed prelabor preterm rupture of the membranes (PPROM) [history of watery vaginal leak and visualization of amniotic fluid leakage on sterile speculum examination].

Group II: (Suspected but unconfirmed PROM group)

- This included 21 preterm pregnant women between 24-36 weeks of gestation who hadj suspected PPROM [history of watery vaginal leak and non-visualization of amniotic fluid leakage on sterile speculum examination].

Group III: (Control groups):

- The control group: included 21 preterm pregnant women between 24-36 weeks of gestation who had intact fetal membranes.

Exclusion criteria:

1. Gestational age below 24 or above 36 weeks.
2. Fetal distress.
3. Any current vaginal bleeding or infection.
4. Placenta previa (because vaginal examination is dangerous, and any microscopic RBCs from placenta previa will affect the results).
5. Any sample contaminated with blood due to abrasion during technique.
6. Patients with PROM and uterine contractions.

Methods:

- Informed consent was taken from every patient and all subjects were provided verbal explanation for the purpose of the study and the method of sample collection.

All pregnant women of the three groups were subjected to:

1-Full history taking:

- Including, the last menstrual period, amniotic fluid leakage (onset, amount, duration, colour of the fluid, etc…)and the history recorded in aspecial chart.

2-General examination:

- Including blood pressure, pulse, temperature, etc….and the results were recorded with the history chart.

3-Blood sample is collected (10 cc of venous blood):

- To do CBC for TLC, ESR and CRP

4-Abdominal examination:

- Including, fundal level, uterine contractions, fetal heart sounds.

5-Transabdominal U/S for:

Gestational age, fetal life, placental site and congenital fetal malformations. and amniotic fluid index calculated by 4 quadrants method, which consists of measuring the vertical diameters of the largest pockets seen in each of the four quadrants of the uterus. The measurements are added, and the result was the amniotic fluid index (AFI).

The AFI is considered normal between 8.1 and 18 cm, low between 5.1 and 8.0 cm, very low ≤5 cm, and high >18 cm. . for optimum accuracy, the mean of three AFI measurements was calculated, when the AFI is <10 cm. ***(Nabhan and Abdelmoula, 2008).***

6-Sample collection:

- Patients laid in lithotomy position in good illumination.
- Sterile vaginal examination using a sterile Cusco speculum was done then vaginal fluid sampling was done as follows:
- Detection of the presence of IGFBP-1 in cervicovaginal discharge by the following technique using the **AMNIOQUICk** kits.

1. The solvent vial is opened and put it in a vertical position.
2. Then collect a sample from the surface of the vagina using a sterile swab.
3. Provided that the polyester tip should not touch anything prior to its insertion into vagina then withdraws the swab from the vagina after 1 minute.
4. Then we place the polyester tip into the vial and rinse the swab in the solvent.
5. Remove and dispose of the swab.
6. Then we dip the white end of the test strip into the vial with solvent. Strong leakage of amniotic fluid results are visible early (within 5 minutes), while a very small leak will take the full 10 minutes.
7. We remove the test strip if two stripes are clearly visible in the vial or after 10 minutes sharp.

If only a control line is visible, the test result is negative. If both control and test lines are visible, the test result is positive. If no lines are visible, the test result is invalid ***(Joong S et al., 2005).***

Amnioquick Test Procedure

Sample Collection

Use the sterile Decron swab to collect secretions on the vaginal surface. Open the swab bag and place the swab into the vagina (5 cm depth) for1minute. Alternatively, speculum may be used and vaginal secretion may be collected by leaving the swab into the vagina (5 cm depth) for 15 seconds.

Step One

Bring the complete kit and samples to be tested to room temperature prior to testing. Open the unit dose vial and lay it vertically on a flat and horizontal surface. Dip the Swap into the unit dose vial and rotate for 10 seconds.

Step Two

2Dip the strip into the vial with arrows pointing downwards. Gently hit dipstick on the bottom of the tube to enhance migration. Keep the strip in the tube in vertical position for 10 minutes.

Step Three

Read the result after 10 minutes from the time the strip is dipped in the tube. Do not interpret any test band appearing 15 minutes after the strip is dipped into the vial.

Principle

The premature rupture of membranes or PROM is relatively frequent and concerns 5 to 10 % of pregnancy cases. It might lead to preterm delivery and fetal infection. The leakage of amniotic liquid is not always detectable by conventional clinical examination and confirmatory biological test is sometimes useful. Biological tests are based on vaginal detection of alkalinization (easy to proceed, sensitive, inexpensive but poor specific) or presence of a molecule which is physiologically present in high concentration in amniotic fluid (diamine oxidase, alpha feto protein, fibronectin, IGFBP-1)

Amnioquick is based on detection of IGFBP-1 which is found in very high concentration in amniotic fluid. Amnioquick can detect less than 1l of amniotic fluid from the collection swab. The test can be used for detection of microrupture or frank rupture of the fetal membranes. Note: In near term women, positive results may appear even in absence of rupture of membranes as the decidual cells of the cervix may release phosphorylated IGFBP-1 that cross react with the test. ***(Akrecan et al.,2005).***

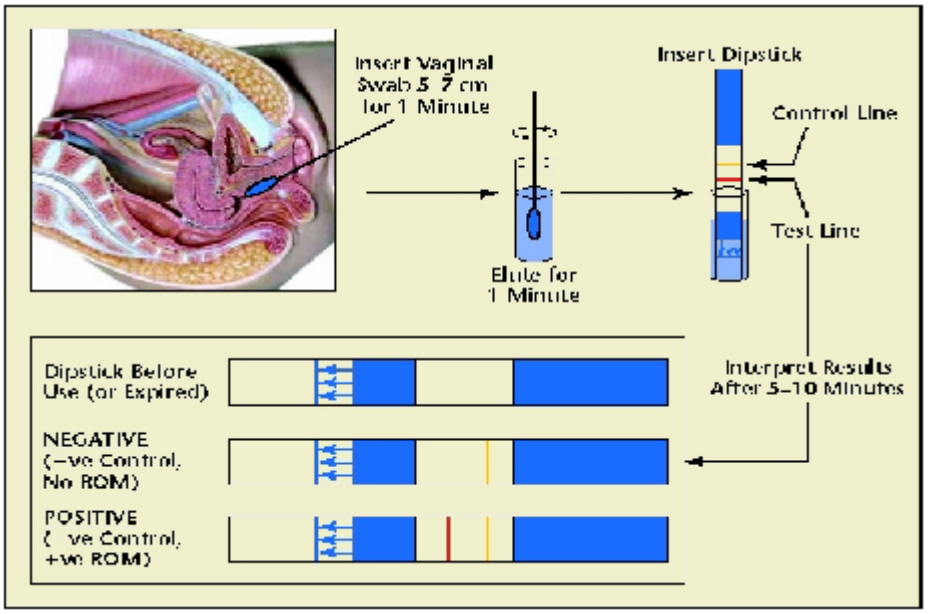

Fig.(7) Amnioquick test for the diagnosis of ruptured fetal membranes.

POSITIVE

Presence of a purple test line (even if intensity is weak) and a purple control line. Presence of amniotic fluid in sample.

NEGATIVE

Absence of purple test line and the presence of a purple control line. Amniotic fluid is not present in sample.

INVALID

A wrong procedure and high viscosity of sample are the two most frequent causes of invalid results. The test has to be run again with a new strip.

Results:

All the data were tabulated and statistically analyzed, using relevant statistical test for comparing three different groups.

Statistical analysis was performed using Microsoft® Excel® version 2010 and Statistical Package for Social Sciences (SPSS®) for Windows® version 15. Data were described as range, mean and standard deviation (for parametric variables); or range, median and interquartile range (for non-parametric variables). Difference between two independent groups was estimated using independent student's t-test (for parametric variables) or Mann-Whitney's U-test (for non-parametric variables). Difference between more than two groups was estimated using one-way ANOVA test (for parametric variables) or Kurskal-Wallis test (for non-parametric variables). Agreement between two variables was estimated using the κ (kappa) coefficient of agreement. Validity of a diagnostic test was expressed in terms of sensitivity, specificity, positive and negative predictive value, likelihood ratio, and overall accuracy. Significance level was set at 0.05.

SUMMARY

Premature rupture of membranes (PROM) is the rupture of fetal membranes at least 24h before onset of labour ,while preterm premature rupture of membranes (PPROM) is the rupture of fetal membranes which proceed the onset of labour for more than 24hours before 37 weeks of gestation ***(Deering et al., 2007).***

Correct diagnosis of PROM has great importance because failure of diagnosis can lead to unwanted obstetric complications ***(Kim et al., 2005).***

The false diagnosis of membrane rupture can lead to inappropriate interventions such as hospitalization or induction of labor. Therefore, many diagnostic test have been discovered including many biochemical markers as B-HCG, urea , creatinine , thyroid hormones fetal fibronectin and IGFBP-1 . ***(Kafali and Oksuzler, 2007)***.

any biochemical test used to establish a correct diagnosis must be reliable, simple and rapid ***(Esim et al., 2003).***

Detection IGFBP-1 in the cervical –vaginal secretions has been shown to be a reliable method in the

diagnosis of rupture fetal membranes in cases in which the clinical diagnosis is uncertain ***(Darj et al.,1998).***

IGFBP-1 detection in cervicovaginal discharge is a new diagnostic test for ROM .it can be used as a marker for diagnosis due to its unique characteristics (i.e., high concentration in the amniotic fluid, low level in blood , and extremely low background level in cervicovaginal secretions with intact fetal membranes) ***(ACOG2007)***.

Insulin like growth factor binding protein is secreted from human liver ,decidual cells and placenta .Its concentration in the amniotic fluid is considerably higher than the concentration in the other body fluids. It is the major Insulin like growth factor binding protein in the amniotic fluid that gradually increases in the second trimester and remains higher throughout pregnancy in comparison to its plasma level ***(Martina et al.,1997)*** .

Amnioquick is the test used for detection of IGFBP-1 in cervicovaginal discharge .

This prospective case-control study was carried out at Ain Shams University Maternity Hospital.

Summary

The study included 63 pregnant women between 24-36 weeks of gestation and were subdivided into three groups of women.

Group I: (Confirmed PROM)

1. Included 21 pregnant women, with uncomplicated pregnancy , between 24-36weeks gestational age, with history of leaking of watery discharge from vagina and visualization of amniotic fluid leakage (sterile cusco speculum examination: positive fluid leak).

Group II: (Suspected but unconfirmed PROM group) Included 21 pregnant women, with uncomplicated pregnancy , between 24-36weeks gestational age, with history of leaking of watery discharge from vagina with no visualization of amniotic fluid leakage (sterile Cusco speculum examination: negative fluid leak).

Group III: (Control groups):

Included 21 pregnant women with uncomplicated pregnancy , between 24-36weeks gestational age, with no history of leaking of any watery discharge from vagina .

All subjects were provided all information about the purpose of the study and the method of sample collection.

Summary

All pregnant women of the three groups were subjected to full history, general examination, abdominal examination, sterile cusco speculum examination and trans abdominal U/S.

- Sterile vaginal examination using a sterile Cusco speculum was done then vaginal fluid sampling was done .

- Detection the presence of IGFBP-1 in cervicovaginal discharge by the previously mentioned technique using the ***Amnioquick*** kits.

It was found that there was no significant statistical difference between the confirmed, suspected and control groups as regard maternal age, parity, gestational age history of vaginal delivery and caesarian section.

After the initial evaluation of the 3 groups, the AmnioQuick dipstick test has 100% sensitivity and 69% specificity. Its positive likelihood ratio was 3.2 and negative likelihood ratio was 0.0. It had 100% agreement with the speculum examination in all cases with evident membrane rupture.