Comparison of the duo of insulin-like growth factor binding protein-1/alpha fetoprotein (Amnioquick duo+®) and nitrazine test for diagnosing query rupture of fetal membranes

Thesis

Submitted for Partial Fulfillment of the Master Degree in Obstetrics and Gynecology

By

Jamila Ahmed Elmabrouk

M.B. B.Ch., 2006 – Tripoli University

Under Supervisors

Prof. Sherif Fekry Abd Allah Hendawy

Professor of Obstetrics and Gynecology

Faculty of Medicine – Ain Shams University

Assist. Prof. Dr. Sherif Hanafi Hussain

Assistant Professor of Obstetrics and Gynecology

Faculty of Medicine – Ain Shams University

Faculty of Medicine

Ain Shams University

2018

سورة البقرة الآية: ٣٢

First of all, thanks to Allah whose magnificent help was the main factor in completing this work.

No words could express my deepest thanks and appreciation to Prof. Dr. Sherif Fekry Abd Allah Hendawy, Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for inspiring me with the idea of this work. His patience, precious advice and guidance enlightened my way throughout this work.

I want also to express my deepest thanks and appreciation to Assist. Prof. Dr. Sherif Hanafi Hussain, Assistant Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for his patience, valuable advice and continuous help in completing this work.

I am also deeply indebted to Dr. Ahmed Al-Anwar, Lecturer of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for his kind help, guidance, useful advices, continuous encouragement and support all through my entire work.

Very special thanks to my family, my respected mother, and my beloved husband for their moral and economic support and assistance in every step of my life.

At the end I would also like to thank to my sweet kids, who suffered a lot with me in all this process.

Dedication

To

Soul of My Great Father

Who Helped Me a Lot and Pushed Me

Forward in Every Step of My Life

May Allah Give Him Mercy

Contents

List of Abbreviations

ACOG : American College of Obstetricians and Gynecologists

AF : Amniotic fluid

AFI : Amniotic fluid index

AFP : Alpha fetoprotein

AFV : Amniotic fluid volume

Alfa-FP : α-fetoprotein

BV : Bacterial vaginosis

CAPs : Contraction associated proteins

DAO : Diamine Oxydase

ECM : Extracellular matrix

FFN : Fetal fibronectin

GBS : Group B streptococcus

HPL : Human placental lactogen

HCG : Human chorionic gonadotropin

HMD : Hyaline membrane disease

IGFBP-1 : Insulin-like growth factor binding protein-1

IGFs : Insulin-like growth factors

IL : Interleukin

MMP : Matrix metalloproteinase

NEC : Necrotizing enterocolitis

NTDs : Neural tube defects

PAMG- l : Placental alpha micro globulin-1

PRL : Prolactin

PROM : Premature rupture of membranes

RDS : Respiratory distress syndrome

ROM : Rupture of membranes

ROS : Reactive oxygen species

S : Streptococcus

SDP : Single Deep Pocket

SNPs : Single-nucleotide polymorphisms

STDs : Sexually transmitted diseases

TCA : Traditional clinical assessment

TIMPs : Tissue inhibitors of matrix metalloproteinases

TNF : Tumor necrosis factor

.

List of Figures

List of Tables

PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRICS AND GYNECOLOGY

Title of the Protocol:

Comparison of the duo of insulin-like growth factor binding protein-1/alpha fetoprotein (Amnioquick duo+®) and nitrazine test for diagnosing rupture of fetal membranes

Postgraduate Student: Jamila Ahmed Elmabrouk
Degree: M.B. B.Ch., 2006 – Tripoli University

DIRECTOR: Sherif Fekry Abd Allah Hendawy
Academic Position: Professor
Department: Obstetrics and Gynecology

Co-DIRECTOR: Sherif Hanafi Hussain
Academic Position: Assistant Professor
Department: Obstetrics and Gynecology

Co-DIRECTOR: Rasha Mahmoud Medhat Abd El-hady
Academic Position: Lecturer
Department: Obstetrics and Gynecology

2017

What is already known on this subject? AND
What does this study add?

Amnioquick duo+® is a rapid strip test with immunoassay that is simple, easy to perform, quick and noninvasive method to diagnosis rupture of membranes.
The aim of this study is to determine accuracy of the duo of insulin-like growth factor binding protein-1/alpha fetoprotein (Amnioquick duo+®) compared to accuracy of nitrazine test for diagnosing query rupture of fetal membranes.

1. INTRODUCTION/ REVIEW

Premature (prelabor) rupture of membranes (PROM) is defined as rupture of the fetal membranes spontaneously prior to the onset of uterine contractions **(Kim et al., 2016).** It is a relatively frequent obstetric phenomenon occurring in 2–18% of pregnancies **(Phupong and Taneepanichskul, 2003).** When it is preterm, it is often associated with prematurity-related complications including premature birth, pulmonary hypoplasia, fetal deformities and infectious materno-fetal morbidity **(Deckmyn et al., 2016)**.

The diagnosis of PROM is straightforward in the presence of obvious rupture of membranes. However, several factors such as urine, vaginal discharge or semen may interfere with traditional clinical assessment (TCA), leading to high levels of false negative and positive results. Such results may lead to inappropriate interventions such as hospitalization and stimulation of labor **(Buyukbayrak et al., 2005; Deckmyn et al., 2016)**. On the other hand, misdiagnosis of PROM may cause providers to withhold appropriate therapy **(Koike et al., 2016)**.

A novel test used for diagnosing rupture of membranes (ROM) is Amnioquick duo+® (Biosynex, Strasbourg Cedex, France). Amnioquick duo+® is a rapid strip test with immunoassay that is simple, easy to perform, quick and noninvasive. It is an immunochromatographic test that identifies even trace amounts of both alpha fetoprotein (AFP) and insulin-like growth factor binding protein-1 (IGFBP-1). The protein markers, though abundant in amniotic fluid, are present in far lower concentrations or undetectable in the maternal blood or in cervicovaginal secretions in the genuine absence of rupture of membranes **(AbuFaza et al., 2016)**.

Thus, such a differential concentration between amniotic fluid and cervicovaginal secretions of the biomarkers makes Amnioquick duo+® an excellent marker for PROM. The test can be performed even when fluid is not obvious in the vagina and its execution does not require laboratory specialized equipment or specially trained personnel **(AbuFaza et al., 2016; Deckmyn et al., 2016)**.

On other hand the traditional methods for diagnosis of PROM are through the patient's history, leakage, ferning test and nitrazine test. The histories told by the patients are

sometimes subjective, and the statements may not be elaborate. The obvious leakage of amniotic fluids from the cervical os can indicate PROM, but we cannot always identify enough fluid to confirm, and sometimes there is no visual leakage in the vagina. The ferning test and the nitrazine test are two generally used methods for diagnosis of PROM. The ferning test has been associated with false positive results in 5–30% of patients and false negative results in 5–12.9% of patients. The nitrazine test is easily contaminated by other fluids, such as semen, urine, blood, and antiseptic solution. PROM is an important dilemma in clinical practice, and we urgently require alternative, accurate and fast methods to help us solve the problem **(Liang et al., 2016).**

2. AIM/ OBJECTIVES

Research hypothesis:
In pregnant women with query PROM, (Amnioquick duo+®) may be accurate as nitrazine test for diagnosis the rupture of fetal membranes.

Research question:
In pregnant women with query PROM, dose (Amnioquick duo+®) accurate as nitrazine test for diagnosis the rupture of fetal membranes?

The aim of this study is to compare the accuracy of the duo of insulin-like growth factor binding protein-1/alpha fetoprotein (Amnioquick duo+®) and nitrazine test for diagnosing query rupture of fetal membranes in pregnant women with query PROM.

3. METHODOLOGY:
Patients and Methods

- **Type of Study:** Evaluation of the accuracy of diagnostic test, cross sectional.
- **Study Setting:** Ain Shams university maternity hospitals.
- **Study Period:** The study will be started at November 2017.
- **Study Population**

Inclusion Criteria:

1. Pregnancy duration of 24 weeks or more.
2. Consenting pregnant women with symptoms, signs or complaints suggestive of membrane rupture.

Exclusion Criteria:

1. Women with vaginal bleeding.

2. Uterine contractions.
3. Fetal anomalies.
4. Placental pathology that could cause oligohydramnios including intrauterine growth restriction.

- **Sample size justification:**

The required sample size has been calculated using the Power Analysis and Sample Size 2008 software version 08.0.15 (PASS© 2008, NCSS, LLC, Keysville, Utah, USA).

A previous study reported that the nitrazine test had a sensitivity of 90.1% and a specificity of 69% for diagnosis of PROM. In contrast, the sensitivity and specificity of Amnioquick duo+® were 97.6% and 97.9%, respectively. That study reported that approximately 73% of women presenting with symptoms suspicious of PROM proved to actually have PROM **(Eleje et al., 2016).**

So, it is calculated that a sample size of 96 women suspected of PROM would include 70 (73%) patients who actually have PROM. This sample achieves 83% power (type 2 error = 0.17) to detect a difference of 8% between the sensitivities of the 2 tests (98% versus 90%) and a power of 100% to detect a difference of 29% between the specificities of the 2 (98% versus 69%) tests using a two-sided binomial test with type I error of 0.05 (i.e., confidence of 95%).

- **Ethical considerations:** written consent will be taken form all participants, confidentiality will be preserved by assigning a number to patient's initials and only the investigator will know it.
- **Study procedures:**
  - ***History taking:***
    - **Personal history:** it includes name, age, occupation, marriage and special habits.
    - **Obstetric history:** it includes parity, last menstrual period, expected date of delivery, gestational age, previous preterm labor and vaginal bleeding.
    - **Medical history:** it includes diabetes mellitus, hypertension and hepatic or renal disease.
    - **Surgical history:** it includes history of previous pelvic or abdominal surgeries.
  - ***Examination:***
    - **General examination:** it includes blood pressure, arterial pulse and temperature.
    - **Abdominal examination:** it includes fundal level, fundal grip, pelvic grip, uterine contractions, tenderness and scars of previous operations.
    - **Local examination:** all women will be put in dorsal lithotomy

position, using a proper light source and sterile gloves; sterile speculum free of gel will placed into vagina.

The Amnioquick duo+ immunoassay (Manufacturer company: Bio-synex) will be performed according to the manufacturer's instructions (Biosynex, 12 rue Ettore Bugatti-CS 28006 67038, Strasbourg Cedex, France). To perform Amnioquick duo+®, a sample of vaginal secretion was collected using a vaginal swab entwined with Sterile Nylon® positioned in the posterior fornix or in the cervical canal for 60 s. Subsequently, the swab was either bathed in a buffer tube for 10 s or the swab tip broken off into the vial buffer and the mixtures shaken together. Three drops of the resultant liquid content were dropped in the specimen well on the cassette (supplied by the manufacturers) stick containing monoclonal antibodies to AFP and IGFBP-I, which absorbs the extracted specimen. The pink line appeared on the A and B zones on the cassette, respectively when the amniotic fluid contained AFP and IGFBP-1. The result was interpreted within 10 min. There were three distinct zones on the cassette for AFP (A), IGFBP-1(B) and control (C). The interpretation of the results was based on the manufacturer's predetermined criteria, classified as positive, negative or doubtful for ROM. The test was positive when the C and the B lines were both present or when the A and the C lines were both present for cases with gestational age ≥ 24 weeks. The test was negative when both the A and the B lines were absent. Lines were evaluated as positive if a continuous line was observable, even if faint **(Liang et al., 2016).**

Nitrazine test (McolorPhastTH):

The pH test strip will be inserted into the posterior vaginal fornix for about 30 s, then removed from the vagina. A blue strip indicated PROM and other colors indicated no PROM.

Statistical Analysis:

Data will be collected, tabulated, then analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY). Normally distributed numerical data will be presented as mean and SD, and skewed data as median and interquartile range. Qualitative data will be presented as number and percentage. Comparison of normally distributed numerical data will be done using the unpaired t test. Skewed data will be compared using the Mann-Whitney test. Categorical data will be compared using the chi-squared test or Fisher's exact test, if appropriate. Diagnostic test accuracy will be examined using ROC curve analysis. P-value <0.05 will be considered statistically significant.

Statistical Package:

Data will be collected, tabulated, then analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY).