Association Between Maternal Serum Level of Alpha-Fetoprotein and Fetomaternal Hemorrhage in High Risk Pregnant Women

Thesis

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By

Amany Nady Abdelaziz Elneairy
M.B.B.,Ch 2010
Resident of Obstetrics & Gynecology
Student hospital in Helwan University

Under Supervision of

Dr. Ahmed Abd-Elkader Fahmy
Professor of Obstetrics & Gynecology
Faculty of Medicine–Ain Shams University

Dr. Ayman Abd-Elkader
Lecturer of Obstetrics & Gynecology
Faculty of Medicine–Ain Shams University

Dr. Rasha Abd El-Rahman El-Gamal
Lecturer of Clinical Pathology
Faculty of Medicine–Ain Shams University

Faculty of Medicine
Ain Shams University
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Abstract

Background: Fetomaternal hemorrhage is considered to be a grave complication which may occur during pregnancy. And due to its graveness the fetomatenal hemorrhage renders the pregnancy as a high risk condition, so it must be diagnosed, followed and managed properly in order to avoid any hazards on the mother and the fetus**.** The Alpha-Fetoprotein is the major protein component of fetal serum which is synthesized by the visceral endoderm of the yolk sac during early fetal life and subsequently by the fetal liver.

Aims: To find an easier and cheaper test for detection and quantification of FMH, with less inter- and intra- observer variation than the standard KBT.

Methodology:

Study type: Diagnostic Test accuracy study.

Study place: In Maternity Hospital at Ain Sham University Hospitals in the period from August 2015 to February 2016, samples were analysed at clinical pathology department at Ain Shams University.

Results: The required sample size had been calculated using the Power Analysis and Sample Size software version 11.0.10 (PASS; NCSS, LLC, Kaysville, Utah). The primary outcome measure is the accuracy of alpha-fetoprotein for diagnosis of significant fetomaternal hemorrhage. A previous study reported that the incidence of significant feto-maternal hemorrhage was of the order of 29%.

Conclusion: Our study showed that around 22% of high risk deliveries have a FMH more than 4 ml which require an extra dose of anti D. And thanks GOD that the high incidence of ABO incompatibility might protect against sensitization but this incidence should certainly ring a bell and raise interest concerning the hazards and bad impact of underestimating the amount of FMH in those populations.

Recommendations: Preoperative sampling for KBT should be done to avoid false +ve results by KBT in patients with persistent Hb F. Exclusion of fetomaternal ABO incompatibility, which yields false – ve KBT.

Keywords: Maternal Serum Level, Alpha-Fetoprotein, Fetomaternal Hemorrhage, High Risk Pregnant Women

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Candidate
Amany Nady Elneairy

Contents

List of Abbreviations

Abbreviation		Full-term
AFP	:	Alpha fetoprotein
AUROC	:	Area under Receiver-operating characteristic curve
CTG	:	Cardiotocography
CVS	:	Chorionic villious sampling
DAT	:	Direct Antiglobin Test
EDTA	:	Ethylenediaminetetraacetic acid
EST	:	Endodermal sinus tumour
FHR	:	Fetal heart rate
FMH	:	Fetomaternal haemorrhage
HDFN	:	Hemolytic disease of the fetus and the newborn
IUD	:	Intrauterine Death
IVT	:	Intravascular intrauterine transfusion
KBT	:	Kleihauer-Betke test
MCA	:	Middle cerebral artery
MoM	:	Multiples of median
MSAFP	:	Maternal serum alpha fetoprotein
NPV	:	Negative predictive value
PBS	:	Phosphate buffered saline
PPV	:	Positive predictive value
PSV	:	Peak systolic velocity
PV	:	Per-vaginal
RAAP	:	Routine antenatal anti-D prophylaxis
RBCs	:	Red blood cells
Rh D	:	Rhesus D
ROC curve	:	Receiver-operating characteristic curve

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Introduction

Fetomaternal hemorrhage is considered to be a grave complication which may occur during pregnancy. And due to its graveness the fetomatenal hemorrhage renders the pregnancy as a high risk condition, so it must be diagnosed, followed and managed properly in order to avoid any hazards on the mother and the fetus ***(Sebring et al., 1990).***

Fetal and maternal circulations are supposed not to be in direct contact. The placental barrier plays the role of separation between them in a way that receiving nutrients by the fetus and gas exchange between mother and fetus are not compromised. That is why the placental abnormalities are the most encountered risk factor in cases that are confirmed to be suffering from fetomaternal hemorrhage ***(Bernstein et al., 1992).***

In normal pregnancy this abnormal contact between the fetal and maternal circulations may occur so late during delivery. Up to 1 ml of fetal blood may pass to the maternal circulation. But in an abnormal pregnancy fetomaternal hemorrhage is defined as the hemorrhage of 30 ml or more of whole blood from a fetus into the maternal circulation. As less than 30 ml is considered to be benign and passes without any remarkable side effects. The fetomaternal hemorrhage was first introduced in 1905 by Dienst who

was doing his researches on what causes the condition of eclampsia; he only described the fetomaternal hemorrhage as presence of fetal blood in the maternal circulation. But he did not have any evidence on that till 50 years later when Chown serologically demonstrated the presence of fetal blood cells in the blood sample of a newly delivered lady ***(Sebring et al., 1990).***

The problem in fetomaternal hemorrhage appears when fetal blood escapes into the maternal circulation in a considerable amount 30 ml or more. That shall compromise the fetal condition leading to many morbidities and mortalities. The result varies from minimal degree of fetal anemia up to severe degree of fetal anemia. And lethal hydrops fetalis may follow that leading to fetal cerebral edema, kernicterus, fluid collection in all body spaces, severe pallor and failed circulation due to the escaping red cells and eventually ending in death of the fetus ***(Zizka et al., 2001).***

Occurrence of fetomaternal hemorrhage is not a predictable event. Even some actual cases are listed to be of unknown cause ***(Bird et al., 1999).***

And also as a clinical wise, most of the fetomaternal hemorrhage cases never produce these evident signs or symptoms by which a confirmed diagnosis can be made upon until it is very late ***(Kecskes, 2003).***

But somehow it might be linked to certain risk factors which if happened to the pregnant lady before in her obstetrical medical history, it gives a clue that this lady in particular is more susceptible for having fetomaternal hemorrhage in her next pregnancies .So more antenatal care and cautious follow up must be given to such lady in order to avoid any complications as possible and also to detect occurrence of the fetomaternal hemorrhage as early as possible so that the management she will be having, could give more optimum outcome ***(Sebring et al., 1990).***

These risk factors include a previous history of spontaneous stillbirth, placental disruption and abnormalities ***(Bernstein et al., 1992),*** anterior position of the placenta, surgical and manual removal of the placenta, twin (monochorionic, monozygotic) pregnancy, premature rupture of membranes, invasive diagnostic procedures as cordocentesis, preterm labor, postmaturity, maternal trauma, external version, vaginal assisted delivery, cesarean section or massive blood loss during pregnancy ***(David et al., 2004).***

The routine investigation used nowadays to detect the occurrence of fetomaternal hemorrhage in pregnant ladies is the standard Kleihauer-Betke test (it was discovered in the 1957) ***(Sebring et al., 1990).***

And it is used in the regular follow up if the diagnosis is already settled especially in all cases of maternal trauma during pregnancy ***(Muench et al., 2004).***

Also another use for the Kleihaeur-Betke test is in calculation of the exact dosage of anti-D which is needed in treatment of cases of Rh alloimmunisation during pregnancy in sensitized Rh negative pregnant ladies (Rh negative mother to Rh positive fetus) ***(Sebring et al., 1990)***.

The principle of the Kleihaeur-Betke test is to do the counting of the approximate number of the escaping fetal red blood cells in the maternal circulation (number of fetal red cells in low power field).The blood sample is dealt by acid elution so maternal red cells appear as ghost cells while the fetal red cells are more resistant and thus appear more easily. And accordingly to the result, the management is chosen ***(Lachman et al., 1977).***

The controversy about Kleihaeur -Betke test appears in being a tedious method which requires a specific laboratory setting and a highly performing clinical pathology specialist so it is not that easy to be conducted and to give reliable results ***(Agarwal et al., 2011).***

As many cases have showed failure of proper calculation of the precise amount of the escaping fetal blood by the Kleihaeur technique. Also there are many

disadvantages to the Kleihauer-Betke test. Firstly it is subjected to the human error in the microscopic manual method and also prolongation of the time between sampling and testing may lead to clotting of the sample and consequently false interpretation in both of the microscopic manual and the microscopic automated methods ***(Lachman et al., 1977).***

Some trials have been recorded on new methods which can detect and quantify fetomaternal hemorrhage. There is flow cytometry method which is detecting fetal red cells in the maternal blood by using anti-fetal red cells antibodies ***(Pelikan et al., 2004).***

Also another trial worked on detecting the genomic material of the nucleated fetal red cells by using molecular biology techniques ***(Hiromi et al., 1995).***

The Alpha-Fetoprotein is the major protein component of fetal serum which is synthesized by the visceral endoderm of the yolk sac during early fetal life and subsequently by the fetal liver. After birth, by passing of time, the high levels of Alpha-Fetoprotein fall rapidly till Alpha-Fetoprotein becomes virtually undetectable by the end of the first year of life ***(Phillip J. Johnson 2001).***