Multiple markers screening to predict fetal chromosomal abnormalities and pregnancy complications

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بسم الله الرحمـن الرحيــم

(وما أوتيتـم من العلـم إلا قليـلا)

صدق اللــه العظيم

الإسراء ٨٥

To study the phenomenon of disease without books is to sail an uncharted sea,
While to study books without patients is not to go to sea at all.

Sir William Osler
Professor of Medicine (1849-1919)

ENGLISH ABSTRACT

Key Words:

(Pregnancy complication, First and second trimester of pregnancy, Multiple marker screening)

The aim

To assess and compare the cost effectiveness of the different strategies for prenatal screening of fetal and pregnancy complication, and to determine the most useful protocol for. And to introduce antenatal rapid aneuplidies detection (ARAD) by QF-PCR test into routine practice to our community.

Methods:

200 pregnant females were included; they were subjected to ultrasound scan for nuchal translucency (NT) nasal bone (NB) and b-hCG at 11-14weeks gestation AND for ultrasound scan for soft markers, major anomaly with maternal serum alpha feto protein (MSAFP) at18 -22weeks and at 26-28weeks

Results: we divided cases into two groups, **group A** who is subjected to NT, NB and β-hCG at 11-14weeks gestation and was 99 cases. **Group B** who is subjected to ultrasound scans for soft markers, major anomaly with maternal serum alpha feto protein (MSAFP) at18 -22weeks and at 26-28weeks and was 193 cases**.** There are 5 cases with increased NT, two cases with decreased free β-hCG**,** three cases with increased free β-hCG, there are 9 cases with decreased MSAFP, amniocentesis was offered to all of them but three cases refused.

Conclusion: The combination of ultrasound and biochemical screening works better than either one alone either in 1st or in 2nd trimester. 2nd trimester ultrasound screening can detect most of the structural abnormalities as NTD, Renal anomalies. QF-PCR is a rapid technique in the prenatal diagnosis of chromosomal aneuploidies. It allows the detection of the most **common** fetal numerical abnormalities with specificity 100% and sensitivity 100%.

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Finally, I am praying **Allah** for the patients who were included in this study. I hope this work may helpful to solve their complaint.

This work is dedicated to my parents, my wife and my beloved sons *Mohammed* and *Al-Hussein* for their endless support. GOD blesses them.

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List of abbreviation

AC	Abdominal circumference
AMXY	Amelogenin locus on X and Y chromosomes
ARAD	Antenatal Rapid Detection
β-hCG	Beta subunit-human chorionic gonadotropin
BPD	Biparietal diameter
CVS	Chorionic villous sampling
CPC	Choroid plexus cyst
CRL	Crown rump length
CI	Cephalic index
DNA	deoxyribonucleic acid
DR	Detection rate
DS	Down syndrome
EDD	Expectd Date of Delivery
EIF	Echogenic intracardiac foci
EMR	Eastern Mediterranean Region
FASTER	First And Second Trimester screening
FL	Femur length
FISH	Fluorescent in situ hybridization
FOD	Fronto occipital diameter
FPR	False positive rate
G banding	Giemsa banding
GH	Gestational hypertension
hCG	Human chorionic gonadotropin
H hCG	Hyperglycosylated Human chorionic gonadotropin
HC	Head circumference
HL	Humerus length
IUGR	Intra uterine growth retardation

IUFD	Intra uterine fetal death
MLPA	Multiplex ligation-dependent probe amplification
MCC	Maternal cell contamination
mmhg	Millimeter mercury
MoM	Multiples of median
MSAFP	Maternal Serum Alfa-fetoprotein
NAD	No abnormality detected
NB	Nasal bone
NOR-staining	Silver staining for nucleolar organizer regions
NTD	Neural tube defect
NT	Nuchal translucency
PAPP-A	Pregnancy associated plasma protein A
PE	Pre-eclmpsia
PI	Pulsatility index
PP13	Placental protein 13
PSG-1	Pregnancy specific Beta1 glycoprotein 1
QF-PCR	Quantitative fluorescent polymerase chain reaction assay
S1	Markers Multiplex set 1
S2	Markers Multiplex set 2
STR	Short tandem repeat
SURRUS	Serum, Urine and Ultrasound Screening Study
TOP	Termination of pregnancy
uE3	Unconjugated estriol
U/S	Ultrasound
WHO	World Health Organization

Introduction

Prenatal screening is the systematic application of a non invasive test to identify fetuses at risk for a disease or a condition before birth to warrant further invasive investigation or direct intervention action. Screening can only evaluate risk of a condition but it cannot determine 100% if the fetus has such condition **(Wald et al., 2004)**.

The international population risk of having a child with congenital abnormality, whether genetically and/or environmentally determined, varies between **3** and **5 % (Bozzette, 2002)**.

Congenital anomalies involving the brain are the largest group at 10 per 1000 live births, compared to heart at 8 per 1000, kidneys at 4 per 1000, and limbs at 1 per 1000. All other somatic anomalies have a combined incidence of 6 per 1000 live births **(Bezerra et al., 2000)**.

The genetic sonogram examination represents a specialized evaluation of the fetus in which the fetus is examined in a detailed manner like a newborn including structural malformations **(Flood and Martin, 2008)**.

First-trimester screening is typically conducted between the 11^{th} and 13thwks 6 days of gestation. At this time nuchal translucency (NT), nasal bone (NB) and free beta subunit of human chorionic gonadotrophin (f ?-hCG) are powerful markers for detection of many maternal and fetal conditions **(Snijders et al., 1998; Cicero et al 2003)**.

Intrauterine growth restriction, unexplained stillbirth, sudden infant death syndrome and placental insufficiency associated with poor obstetric and neonatal outcomes, can be screened and predicted prenatally via f ?-hCG

in the 1st trimester **(Sahota et al., 2009; Wortelboer et al., 2009)**.

The advantage of this test is the availability of the results in the late first trimester with early surgical termination of pregnancy when this is indicated. Thus the American College of Obstetrician and Gynecologist suggested that 1st trimester testing should be routine for general population screening as a method of detecting genetic disorders. **(ACOG, 2007)**.

Second trimester biochemical markers represent part of physiological biochemical changes during pregnancy that couldbe affected by pathological pregnancies and may be monitored for diagnosis or prediction, and management of pregnancy complications (**Fernando et al., 2002; Peter et al., 2002)**.

Alpha feto protein (AFP) is the best and most effective marker for detection of open neural tube defect. It is a simple test that achieves high detection rate with low cost **(Platt et al., 2004).**

AFP serum levels have been used in the screening of chromosomal abnormalities and also as an aid in the diagnosis of placental abnormalities and disorders like fetal death and growth restriction **(Bartha et al., 1999).**

In a study carried by **Kang and his colleagues (2008);** they found that Down syndrome biochemical markers like maternal serum alpha feto protein (MS-AFP); (free ?-hCG) levels were altered in patients who subsequently developed pre-eclampsia and may be a useful screening test for pre-eclampsia.

Ultrasound exam with serial free ?-hCG testing can predict miscarriage. Prospective studies using very sensitive early pregnancy tests have found

that 25% of pregnancies are miscarried by the sixth week since the woman's Last Menstrual Period LMP **(Wilcox et al., 1999)**.

Clinical miscarriages (after the sixth week LMP) occur in 8% of pregnancies **(Wang et al., 2003)**.The risk of miscarriage decreases sharply after the 10th week LMP, i.e. when the fetal stage begins. The loss rate between 8.5 weeks from LMP and birth is about 2% and this test should be offered to every female with low cost and high prediction rate **(Rodeck and Whittle, 1999)**.

The value of prenatal screening in predicting fetal and maternal complications has been proved as an important part of routine antenatal care **(Filkins and koos, 2005).**

Prenatal diagnosis of pregnancies at increased risk of a chromosomal abnormalities are carried out by karyotype analysis of cultured fetal cells by amniotic fluid sampling , with reporting time (12-21days) for completion, which make more anxiety on the parents. Quantitative fluorescent polymerase chain reaction (QF-PCR) analysis is now recognized as a viable complimentary to cytogenetic analysis for the rapid detection of aneuploides. Usually within 48 – 72 hours **(Shaffer and Bui, 2007)**.

QF-PCR has recently entered the field of prenatal diagnosis to overcome the need to culture fetal cells, hence to allow rapid diagnosis of some selected chromosomal anomalies. The detection rate of aneuploidies of the selected numerical chromosomes (13, 18 and 21, and X and Y) is 98.6 **(Umberto et al., 2004).**