Quantitative Analysis Of Fetal DNA In Maternal Conditions Associated With Placental Abnormalities

Thesis
Submitted in Partial Fulfillment of Master Degree in Obstetrics & Gynecology

By

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List of Abbreviations

AFP	Alpha feto-protien
APAS	Anti phospholipid antibody syndrome
BMI	Body mass index
BPs	Binding protiens
ccf DNA	Circulating cell free fetal DNA
CEA	Carcino embryonic antigen
cT	Threshold cycle
DM	Dystrophia myotonica
DMPK	Dystrophia myotonica protein kinase
FGFR٣	Fibroblast growth factor receptor gene ٣
FGR	Fetal growth restriction
FISH	Flourescence in situ hybridization
HCG	Human chorionic gonadotrophin
HEG	Hyperemesis gravidarum
HELLP	Hemolysis, elevated liver enzymes, low platlets count
IGF١	Insulin growth factor ١
IQR	Interquartile range
IUGR	Intrauterine growth retardation
NHD	Neonatal hemolytic anaemia
NIPD	Non invasive prenatal diagnosis
NPV	Negative predictive value
NRBCs	Nucleated red blood cells
PCR	Polymerase chain reaction
PIH	Pregnancy induced hypertension
PPV	Positive predictive value
SD	Standard deviation
SLE	Systemic lupus erythromatosus
TUNNEL	Terminal Udtp nuclear end labeling

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INTRODUCTION

Prenatal diagnosis of inherited disorders is mainly based on genetic testing of fetal materials obtained from amniocentesis or chorionic villous sampling. These invasive procedures carry a significant risk for both fetus and mother (Evans& Andriole, ٢٠٠٨; Forabosco et al., ٢٠٠٩), which burdens not only the affected pregnant women but also their family members (Grant& Yagel, ٢٠٠١; Odibo et al., ٢٠٠٨; Cohen& Yagel, ٢٠٠٩). The discovery of circulating cell-free fetal DNA in maternal plasma opens a great hope for risk-free noninvasive prenatal diagnosis (NIPD) (Hahn et al., ٢٠٠٧; Lo, ٢٠٠٩).

Lo et al. (١٩٩٨) demonstrated the presence of circulating cell- free fetal (ccf) DNA in the maternal circulation, which offered an approach for non invasive prenatal diagnosis (NIPD) (Redman& Wainscoat, ١٩٩٧; Zhong et al., ٢٠٠١). In addition, high levels of ccf DNA in plasma and serum samples have been widely described in a variety of clinical pathological conditions such as pathological pregnancies, inflammatory diseases and trauma (Lederrey& Stroun, ٢٠٠١; Lo, ٢٠٠١). These findings suggest that ccf DNA may help in understanding the biological nature of disease and be of use in clinical diagnostic applications (Anker& Stroun, ٢٠٠١; Stroun& Anker, ٢٠٠٥; Lo, ٢٠٠٨).

Circulating cell free DNA has been found in the plasma of human subjects (Wochenschr, ١٩٧٩; Clin, ١٩٨٠). Recent applications of molecular biologic techniques have allowed the molecular characterization of circulating DNA in certain pathologic and physiologic conditions. Thus, tumor associated genetic changes have been found in the plasma and serum of patients suffering from a

number of cancers (Nat Med ١٩٩٦; Clin, ١٩٩٨). After solid organ transplantation, donor-derived DNA has also been found in the plasma of the recipients (Lancet ١٩٩٨). Physiologically, fetal derived DNA has been found in the plasma and serum of pregnant women (Lancet, ١٩٩٧). Taken together, these studies indicate that circulating non host DNA is a common phenomenon in many clinical and physiological scenarios.

Lo et al. (١٩٩٧) stated that the rapidly growing fetus and placenta possessed tumor like qualities. Due to much similarity between the placenta and malignant tumor, some investigators termed the placenta "pseudo-malignant"(Strickland& Richard, ١٩٩٢). Such a similarity suggests that just as tumor DNA can be found in the circulation of cancer patients, fetal DNA may also be found in maternal plasma.

Because invasive techniques of prenatal diagnosis such as amniocentesis and chorionic villous sampling are expensive and associated with risks to mother and fetus, alternatively non invasive methods are being actively explored (Haematol, ١٩٩٩).

The presence of extra-cellular fetal DNA in maternal plasma has recently been demonstrated (L٠ et al., ١٩٩٨). Little is known about the physiological or pathological factors affecting its release into and clearance from the maternal circulation. It has been observed that fetal DNA concentration in maternal plasma increases in pregnancy complications associated with placental abnormalities, such as hypertension and hypertensive disorders (Lo et al., ١٩٩٨). This might probably be attributed to enhanced transplacental cell transfer and increased cell destruction, which might affect liberation, clearance or turnover of circulating DNA.

In contrast to the traditional teaching that the placenta forms an impermeable barrier between the pregnant women and her fetus, multiple studies show that both intact fetal cells and cell free nucleic acids circulate freely within maternal blood (Haematol, ١٩٩٩).

The feto-maternal transplacental cell trafficking seems to be enhanced in pregnancies complicated by hypertensive disorders, where an increased flow of nucleated fetal cells (trophoblasts and erythroblasts) into maternal circulation has been demonstrated (Walknowska et al., ١٩٦٩; Lo et al., ١٩٨٩).

Hypertensive disorders is a serious pregnancy complication affecting ٥٪-٧٪ of pregnancies and presented with high blood pressure and protinuria (Levine et al.,١٩٩٨). Frequently, this condition may also be associated with intrauterine growth retardation (IUGR). The HELLP (hemolysis, elevated liver enzymes, low platelets count) syndrome is considered to be a severe form of hypertensive disorders having an incidence between ٠,٣٪- ٠,٨٪, which is considerably lower than that of hypertensive disorders (Weinsten, ١٩٨٢).

The pathogenesis of these disorders is not completely understood, but the primary common pathological pathways seem to be a failure of trophoblastic invasion of the spiral arteries, resulting in placental insufficiency (Robertson et al., ١٩٨٦), which is associated with widespread apoptosis of cytotrophoblasts that invade the spiral arteries of the uterus (Difederico et al., ١٩٩٩). In this regard, programmed cell death is one of the proposed mechanisms leading to fetal DNA release in maternal plasma. Presence of the fetal DNA in the maternal circulation results from the constant active remodeling of the placenta, with ongoing constant apoptosis and replacement of the placental cells at the maternal-fetal interface and

trans placental leakage of the fetal cells (trophoblast , lymphocyte , granulocyte , nucleated red blood cells and stem cells) Fetal DNA becomes detectable from the fifth month of the pregnancy (Clin, ٢٠٠١).

These conditions remain a major cause of fetal and maternal morbidity and mortality (Mies et al., ١٩٩٣). The danger of these pathologies is exacerbated by the fact that the maternal and fetal pathological signs can suddenly appear at any time from mid-second trimester until term. Despite improvement of criteria to identify the women at risk, no test is truly predictive of later development of these pathologies (Thom et al., ١٩٩٥). Understanding whether abnormal patterns of fetal DNA may be detectable, even before the manifestation of clinical signs of these disorders, might result in a powerful test for the risk of developing these pathologies and allow one to adopt preventive strategies.

Lo et al., (١٩٩٧) looked for fetal DNA in maternal plasma and serum, using the detection of Y- chromosome DNA sequences derived from a male fetus as a model system. This work produced the first demonstration of fetal DNA in maternal plasma and serum and realization that maternal plasma DNA is a chimeric mixture of fetal and maternal DNA. Before the demonstration of this chimerism, plasma and serum were discarded by investigators looking for nucleated cells in the maternal circulation (Clin, ١٩٩٩; Houfflin et al., ٢٠٠٠).

Using commercial column- based DNA extraction methods and sensitive real-time PCR technique, sensitivity close to ١٠٠٪ has been reached for the detection of a fetal- derived Y- chromosomal sequence from maternal plasma and serum (Lo et al., ١٩٩٨). Using a sensitive tested- PCR protocol, Smid et al. (١٩٩٩) was able to obtain very reliable detection of Y- chromosomal targets from maternal

plasma in women carrying male fetuses. In addition to improving the sensitivity of detection, the real time PCR system can be used to measure the concentration of fetal DNA in maternal plasma and serum (Lo et al., ٢٠٠٠).

Between the ١١th and the ١٧th week of pregnancy the fetal DNA in the serum accounts for the ٣,٤٪of the total maternal DNA, gradually rising to reach the level of approximately ٦,٢٪ between the ٣٧th and ٤٠th week of pregnancy. After birth, its concentration decreases rapidly, as a result of the increased renal filtration.

We aimed at this study to analyze the presence of fetal DNA expressed in copy numbers in the plasma of pregnant women with hypertensive disorders and to show that increased fetal DNA concentration represents a valuable marker for placental abnormalities. This rise in DNA may precede clinical manifestation of hypertensive disorders by few weeks.

Aim of the work

١. To analyze the presence of fetal DNA expressed in copy numbers in the plasma of pregnant women with hypertensive disorders complicated pregnancy.

٢. To prove that increase fetal DNA concentration represents a valuable marker of placental abnormalities. This rise in DNA may precede clinical manifestation of pre-eclampsia by few weeks.

٣. This study allows one to adopt preventive strategies to avoid complications.