PREDICTIVE VALUE OF CELL-FREE FETAL DNA CONCENTRATION IN MATERNAL PLASMA IN PREGNANCIES ASSOCIATED WITH ABNORMAL UTERINE PERFUSION

Thesis
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List of Abbreviations

ALT:	Alanine aminotransferase test
AST:	Aspartate aminotransferase test
BP:	Blood pressure
CI:	Confidence Intervals
DNA:	Deoxyribonucleic Acid
FVW:	Flow velocity waveform
IUFD:	Intrauterine fetal death
IUGR:	Intrauterine growth restriction
Maspin:	Mammary serine protease inhibitor
NIH:	*National Institutes of Health*
P.I.:	Pulsatility Index
PCR:	Polymerase Chain Reaction
R.I.:	Resistance Index
S/D:	Systolic/Diastolic
SGA:	Small for Gestational Age
SRY:	Sex determining Region of Y chromosome
Taq:	Thermus aquaticus bacteria.

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Introduction

Preeclampsia is a pregnancy-related syndrome, characterized by maternal hypertension and proteinuria, with complications ranging from 2 to 10% of all pregnancies. The early onset of preeclampsia has been shown to be associated with a severe risk of perinatal morbidity and mortality for mother and child ***(Roberts, 1994)***.

During normal pregnancy, trophoblastic invasion of uterine spiral arteries takes place reducing the vascular resistance and allowing adequate fetoplacental blood supply. In preeclampsia this adaptive phenomenon is often insufficient, resulting in a diminished infiltration and modification of the spiral arteries, which lead to the maintenance of a high-resistance uterine circulation ***(Lyall, 2002)***.

Impeded blood flow in the uterine arteries can be revealed by uterine artery Doppler velocimetry ***(Campbell et al., 1983)***. Uterine artery Doppler abnormities were correlated with specific disorders of pregnancy, including gestational hypertension, pre-eclampsia, and intrauterine growth restriction (IUGR) ***(Martin et al., 2001; Schuchter et al., 2001; Papageorghiou et al., 2001)***. Such a method was tested in both low-risk and high-risk pregnancies ***(Goffinet et al., 2001; Chien et al., 2000)***, and several studies report results

obtained using, for example, specific resistance index (RI) cut-off points ***(McCowan et al., 2001; Coleman et al., 2000)***. The resistance index is calculated as (systolic velocity–diastolic velocity/systolic velocity).

The pathogenesis of preeclampsia remains unclear. However, it is known that placental dysfunction initiates the systemic symptoms and delivery resolves the condition, suggesting that the placenta plays a central role in the pathogenesis of preeclampsia ***(Sankaralingam et al., 2006)***.

In preeclampsia, poor placental perfusion leads to placental ischaemia. It has been proposed that the aged nuclei and nuclear materials are apoptoticnecrotically liberated and shed as cell-free debris from the ischaemic placenta into the maternal circulation. In normal pregnancy, these cell debris are apoptotically liberated and delivered as membrane enclosed structures into the maternal blood stream ***(Hahn et al., 2005)***.

The cell-free apo-necrotic debris could possibly alter endothelial and vascular function and cause widespread intravascular inflammatory response, which is a typical symptom of preeclampsia ***(Redman et al., 1999)***.

Bi-directional fetomaternal trafficking of nucleic acids has physiologic and clinical implications for noninvasive prenatal diagnosis. Several recent papers describe the

concentration of cell-free fetal DNA found in maternal plasma associated with normal pregnancies bearing a male fetus. Other studies report concentrations of cell-free fetal DNA found in maternal plasma associated, with pregnancies complicated by fetal aneuploidies ***(Lo et al., 1999)*** and/or pregnancies complicated by disorders such as hypertension and preeclampsia ***(Smid et al., 2001; Leung et al., 2001)***. In addition, elevation of cell-free fetal DNA in maternal circulation has been demonstrated in pregnancies complicated by hyperemesis gravidarum ***(Sekizawa et al., 2001)***, polyhydramnios ***(Zhong et al., 2000)***, pre-term labour ***(Leung et al., 1998)***, invasive placenta ***(Sekizawa et al., 2002)***, and HELLP syndrome ***(Smid et al., 2001; Swinkels et al., 2002)***. These results, even if obtained from different populations, basically agree with the observation that abnormal quantities of fetal DNA are related to complications in pregnancy. Because of these observations, the possible clinical use of cell-free fetal DNA for clinical screening of complications related to pregnancy, including preeclampsia, gestational hypertension, intrauterine growth restriction, and intrauterine fetal death, is a matter of current discussion.

Aim of the Work

• Study objectives:

In this study, considering that the hypertensive disorders of pregnancy has multifactorial origins which involve fetoplacental and angiogenic factors, the combination of different tests represents a promising avenue for the early detection of these disorders. Based on the current knowledge, we propose to investigate combinations of uterine artery Doppler analysis, and cell free fetal DNA as predictive indexes in our screening method.

• Hypothesis:

The combination of uterine artery Doppler, cell free fetal DNA will provide a sensitive and specific strategy for an early prediction of the hypertensive disorders of pregnancy.

• Objectives:

- ✧ **Primary:** to evaluate the role of maternal plasma cell free fetal DNA concentration in prediction of preeclampsia in normotensive pregnant women with abnormal uterine artery Doppler velocimetry.
- ✧ **Secondary:** We also evaluate its role in prediction of other pregnancy complications related to poor placental perfusion such as gestational hypertension, intrauterine growth restriction.

Hypertensive Disorders in Pregnancy

• Classification of hypertensive disorders in pregnancy

The classifications that focus on diastolic blood pressure or its changes are so much. There is a great variation in clinical expression of the syndrome and also difficulty to distinguish clinical situation induced by pregnancy from underlying (but often latent) maternal symptoms, and so we have difficulty to define the symptoms, clinical forms, and the pathophysiology that becomes more complex because every time there is new evidence found. Indeed, there is a great confusion and controversy about the criteria used to identify the disorder. Clearly, presence of reliable and easily obtainable classification is needed to improve the clinical issues (prognostics and decision making) and to enable comparison of research work. American College of Obstetricians and Gynecologists (ACOG) has recommended a classification to define hypertensive disorders of pregnancy. This classification is widely accepted and consists of four terms as follow: ***(ACOG Practice Bulletin 2001)***

1) Chronic hypertension:

Chronic hypertension is defined as hypertension that is present and observable before pregnancy or that is diagnosed before the 20th week of gestation. *Hypertension* is defined as a blood pressure equal to or greater than 140 mm Hg systolic or 90 mm Hg diastolic. During pregnancy the hypertension remains, but proteinuria does not occur.

Women who develop hypertension during pregnancy, without proteinuria or seizures, and whose blood pressure remains elevated after pregnancy are also diagnosed with chronic hypertension.

Hypertension that is diagnosed for the first time during pregnancy and that does not resolve postpartum is classified as chronic hypertension too.

2) Pre-eclampsia / Eclampsia:

Preeclampsia is characterized by blood pressure greater than or equal to 140 mm Hg systolic or 90 mm Hg diastolic occurring after midpregnancy (20 weeks gestation), and accompanied by proteinuria. Preeclampsia may be further categorized as mild or severe.

If there is no proteinuria the disease is suspected when elevation of the blood pressure is accompanied by the

symptoms (headache, blurred vision, and abdominal pain), or with abnormal laboratory tests (specifically, low platelet counts and abnormal liver enzymes).

Gestational blood pressure elevation should be diagnosed on the basis of at least two determinations. Repeating of blood pressure measurment should be performed in a manner to reduce the likelihood of artifact and/or patient anxiety.

Proteinuria is defined as the urinary excretion of 0.3 g protein or greater in a 24-hour specimen. This will usually equivalent to 30 mg/dL ("1+ dipstick") or greater in a random urine sample, but evidence of urinary tract infection should be absent. However, due to the discrepancy between random protein determinations and 24-hour urine protein in preeclampsia (which may be either higher or lower), the diagnosis should be based on a 24-hour urine if at all possible or a timed collection corrected for creatinine excretion.

According to the severe of syndrome, ACOG made three categories for preeclampsia:

A. Mild preeclampsia

- Blood pressure (BP) 140/90
- 300mg of proteinuria in 24hrs