Relation between Plasma Cell Free Fetal DNA level and uteroplacental blood flow in pre-eclamptic Women

Protocol of Thesis Submitted for Fulfillment of Master Degree in Obstetrics and Gynecology

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(وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ)

صدق الله العظيم

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Abstract

The purpose of this study to evaluate the cffDNA level in maternal venous samples obtained from pre-eclamptic pregnant women and whether it shows a correlation with uteroplacental blood flow or not. One hundred and twenty pregnant women during third trimester who attended Cairo university hospital (El Kasr El Aini) participated in this cross sectional study between January (2011) – January (2012). Maternal venous samples were obtained from them for cffDNA extraction. Sixty samples were obtained as control from normotensive pregnant women and sixty from pre-eclamptic women. Doppler U/S including umbilical artery RI, uterine artery RI, PI and MCA / Umb PI ratio was done to these pregnant women.

Key Words :

DYS 14 - RASSF1A - RAR B2 .

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Abbreviations

No.		
1.	cffDNA	Cell Free Fetal DNA
2.	RASSF1A	RAS-associated domain family 1
3.	RI	Resistant Index (peak systolic flow – end diastolic flow / peak systolic flow)
4.	PI	PulsatilityIndex (peak systolic low – end diastolic flow / mean flow)
5.	SRY	Sex-determining Region Y " sex-determining gene on Y chromosome"
6.	DYS 14	DYS 14 marker located on Y chromosome "Multicopy sequence of DYS 14 on Y chromosome"
7.	DYZ 3	DYZ 3 marker located on Y chromosome "Multicopy sequence of DYZ 3 on Y chromosome"
8.	CD 34	It's a cell surface glycoprotein & function as a cell-cell adhesion factor.
9.	CD 58	It's a cell adhesion molecule expressed on Antigen Presenting Cells (APC) particularly macrophage
10.	RAR B2	Retinoic Acid Receptor B2
11.	UdTP	Uridine deoxy Tri Phosphate

Introduction

Introduction

Hypertension is a common medical complication during pregnancy, pre-eclampsia belongs to a group of hypertensive disorders in pregnancy that can be divided into gestational hypertension, chronic hypertension, pre-eclampsia, and pre-eclampsia superimposed on chronic hypertension (Brown et al., 2001). Pre-eclampsia occurs in 2-5% of pregnancies but complicates up to 10% of pregnancies in developing countries, where emergency care is often inadequate or lacking (Simon et al ., 2009). The incidence is 4.1% in the first pregnancy and 1.7% in later pregnancies overall, however the risk of recurrence is 14.7% in the second pregnancy for women who had pre-eclampsia in their first pregnancy and 31.9% for women who had pre-eclampsia in the previous two pregnancies (Sonia et al., 2009). Recurrence of pre-eclampsia is influenced by many factors, including the presence of underlying illness, genetic tendency, and change of partners (Sibai et al., 1996).

Severe pre-eclampsia is associated with significant maternal morbidity, including eclamptic seizures, intracerebral haemorrhage, pulmonary oedema or heart failure, acute renal failure, liver dysfunction, and coagulation abnormalities. Fetal complications include abruptio placentae, intrauterine growth restriction, premature delivery, and intrauterine fetal death (Douglas et al., 1994), with 0.4% maternal mortality rate (Douglas et al., 1994) and10-15% in the developing countries (Asia, Africa, Latin America and the Caribbean)(Duley et al., 1992).

One of the main aims of routine antenatal care is to identify mothers or babies at risk of adverse outcomes; Doppler ultrasound is a part of antenatal

care which uses sound waves to detect the movement of blood in vessels. It studies blood circulation in the baby, the mother's uterus and the placenta. Medical interventions may improve outcomes in cases of abnormal blood circulation (Stampalija et al., 2010).

Assuming that defective placental circulation results in adverse pregnancy outcome, Doppler ultrasonography has been used as a modality to evaluate placental circulation and fetal well being for about three decades (Divon et al., 2001). Abnormal development of placental vasculature is considered as the pathophysiological basis for development of pre-eclampsia (Cunningham et al., 2005**)** and this could be reflected in abnormal umbilical Doppler velocimetry. In normal pregnancies, the feto-placental circulation acts as a low resistance system unit. Thus, the blood velocity waveforms in umbilical artery (UA) show continuous forward flow throughout the cardiac cycle (Divon et al., 2001).

High-resistance uterine artery Doppler in the third trimester of pregnancy is able to predict adverse postpartum outcome (De Melo et al., 2010). Women with late-onset pre-eclampsia show a higher risk of perinatal complications if uterine resistance is increased although maternal outcome does not seem to be related to Doppler findings (Ghi et al., 2009). Doppler waves of middle cerebral artery (MCA) can predict most of fetuses in high risk pregnancies **(**Tarzamni et al., 2008**).** Several studies have shown the efficacy of the middle cerebral artery (MCA) Doppler assessment during pregnancy (Gramellini et al., 1992; Bahlmann et al., 2002).

Cell free fetal DNA in maternal plasma has opened up new possibilities for non invasive prenatal diagnosis of sex-linked disorders (Costa et al., 2002),

fetal rhesus D (RhD) status (Bianchi et al., 2005) and β-thalassemia (Chiu et al., 2002).

Several studies reported that in patients with pre-eclampsia, the maternal plasma concentration of cell free fetal DNA (cffDNA) using a Y-chromosomal DNA sequence is increased from 2 to 15 fold higher than in normotensive control subjects (Lo et al., 1999; Crowley 2007). It has been postulated that impaired trophoblastic invasion of the maternal spiral arteries leads to placental ischemia with release of necrotic or apoptotic syncytiotrophoblast fragments that contain fetal DNA into the maternal circulation (Levine et al.,2004; Farina et al.,2004). In addition to evidence for increased entry of cffDNA into the maternal circulation, there is also evidence for reduced clearance of cffDNA from maternal plasma (Lau et al., 2002). There is controversy whether the altered levels precede the onset of the disease or not (Leung et al., 2001; Crowley 2007).

Recently, the promoter of the *RASSF1A* tumor suppressor gene **(**RAS-association domain family 1) has been demonstrated as hypermethylated in the placenta but hypomethylated in maternal blood cells**.** Consequently, the background maternally derived hypomethylated *RASSF1A* sequences could potentially be removed by methylation-sensitive restriction enzyme digestion, whereas the hypermethylated placental (fetal) *RASSF1A* sequences are resistant to methylation-sensitive restriction enzyme digestion and thereby should be detectable and quantifiable by real-time PCR (Lum et al., 2010). The quantitative measurement of a fetal DNA target that could be similarly detected in both male or female pregnancies is useful for monitoring and predicting pregnancy-related conditions associated with aberrant fetal DNA concentrations, including pre-eclampsia (Lo et al., 1999) and certain fetal chromosomal aneuploidies (Zhong et al., 2000).

Aim of the work

The purpose of this study to evaluate the cffDNA level in maternal venous samples obtained from pre-eclamptic pregnant women and whether it shows a correlation with uteroplacental blood flow.