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Cell-free Nucleic Acids as Potential Markers for Preeclampsia

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ABSTRACT

Preeclampsia is one of the leading causes of maternal and fetal/neonatal mortality and morbidity worldwide. Therefore, widely applicable and affordable tests are needed to make an early diagnosis before the occurrence of the clinical symptoms. Circulating cell-free nucleic acids in plasma and serum are novel biomarkers with promising clinical applications in different medical fields, including prenatal diagnosis.

Quantitative changes of cell-free fetal (cff)DNA in maternal plasma as an indicator for impending preeclampsia have been reported in different studies, using real-time quantitative PCR for the male-specific *SRY* or *DYS 14* loci. In case of early onset preeclampsia, elevated levels may be already seen in the first trimester. The increased levels of cffDNA before the onset of symptoms may be due to hypoxia/reoxygenation within the intervillous space leading to tissue oxidative stress and increased placental apoptosis and necrosis. In addition to the evidence for increased shedding of cffDNA into the maternal circulation, there is also evidence for reduced renal clearance of cffDNA in preeclampsia. As the amount of fetal DNA is currently determined by quantifying Y-chromosome specific sequences, alternative approaches such as the measurement of total cell-free DNA or the use of gender-independent fetal epigenetic markers, such as DNA methylation, offer a promising alternative. Cell-free RNA of placental origin might be another potentially useful biomarker for screening and diagnosis of preeclampsia in clinical practice. Fetal RNA is associated with subcellular placental particles that protect it from degradation. Its levels are ten-fold higher in pregnant women with preeclampsia compared to controls. In conclusion, through the use of gender-independent sequences, the universal incorporation of fetal nucleic acids into routine obstetric care and into screening or diagnostic settings using combined markers may soon become a reality. Effort has now to be put into the establishment of standardized and simplified protocols for the analysis of these biomarkers in a clinical setting.

1. Introduction

Preeclampsia is one of the leading causes of maternal and fetal/neonatal mortality and morbidity worldwide [1]. The disease occurs in 2–5% of pregnancies in the Western countries, but it complicates up to 10% of pregnancies in the developing world, where emergency care is often inadequate or even lacking. Therefore, there is a need for widely applicable and affordable tests which can identify women at risk early in pregnancy and subsequently monitor them throughout pregnancy, and thus provide the best prenatal care for these patients and their children. Furthermore, if these tests could provide useful indications as to which women are likely to develop early onset preeclampsia or a severe form of the disorder, this would allow an accurate risk categorization of women and would permit medical care providers to plan a more tailored course of action, e.g. a referral to a specialist centre. This single action alone decreases neonatal mortality by approximately 20% [2].

Currently, there is no single reliable parameter for the prediction of preeclampsia, and much attention has turned towards developing non-invasive testing methods, including ultrasound examination and the quantification of various blood-borne and urinary biomarkers.

The discovery of fetal cells and later of cell-free fetal (cff)DNA [3] in maternal blood opened a new perspective in the field of noninvasive prenatal diagnosis [4–6]. To date, two applications of cffDNA, namely gender determination for X-linked genetic disorders and the prenatal diagnosis of the fetal Rhesus D status, have already translated into clinical routine [7–9]. Furthermore, it is believed that circulating fetal cells and cffDNA in the maternal circulation may provide indirect clues to the underlying physiology and eventual pathology of the feto-placental unit during all trimesters.

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2. Methodology

Relevant articles for this review were identified by a search of electronic information including the PubMed and Embase databases using the following search terms: "preeclampsia"; "noninvasive prenatal diagnosis"; "cell-free fetal DNA"; "total cell-free DNA"; "cell-free fetal RNA". Articles were expertly reviewed and selected, with particular focus on those articles reporting measurement of cell-free fetal DNA, total cell-free DNA and fetal RNA levels in plasma or serum in women with preeclampsia, or in women destined to develop preeclampsia, versus matched normotensive patients.

3. Noninvasive prenatal diagnosis with cell-free fetal DNA from maternal blood

After the initial description of the presence of placental cells within the lungs of patients who deceased from preeclampsia in 1893 by Georg Schmorl [10], several groups reported many decades later that fetal-derived cells such as trophoblasts [11] and fetal erythroblasts [12] were increased in the maternal circulation in this pregnancy condition. Subsequently, similar findings were observed for cell-free nucleic acids.

The first report of quantitative changes in cffDNA in maternal plasma of preeclamptic patients was made by Lo et al. in a study comparing 20 preeclamptic women and 20 gestational age matched controls from the third trimester [13]. The levels of cffDNA, which were measured using real-time quantitative PCR for the *SRY* gene locus on the Y-chromosome were increased approximately 5-fold in women with preeclampsia compared to the normotensive controls (381 versus 76 genome-equivalents/mL, $P < 0.001$). The levels of total cell-free DNA circulating in the maternal blood, which were measured by quantifying the ubiquitous glyceraldehyde-3-phosphate dihydrogenase (*GAPDH*) sequence or the *beta-globin* gene, were also increased in this pathology [14,15].

Leung et al. could demonstrate in a subsequent study, using blood samples from 18 patients who later developed preeclampsia versus 33 controls, that cffDNA levels in maternal plasma were already significantly increased before the onset of the clinical symptoms [16]. This initial finding was confirmed by Zhong et al. using blood samples from women before the onset of clinical symptoms in the second trimester [17]. Using the same assay as Lo et al. they showed a significant difference of circulating cffDNA levels before the onset of clinical symptoms (median 422.9 versus 128.5 copies/mL; $P = 0.005$).

The largest study to date in that field was conducted by Levine et al. using stored samples of 120 women who developed preeclampsia and 120 controls and who underwent uneventful pregnancies, which were collected for the *Calcium for Preeclampsia Prevention trial cohort of healthy nulliparous women* [18,19]. This study showed a two- to five-fold increase of cffDNA levels, starting from week 17 of gestation, in women who subsequently developed the disorder compared to the gestational age matched controls. In addition, the authors demonstrated that the elevation of cffDNA was bi-phasic. An initial elevation was seen between 17 and 28 weeks of gestation, and the second elevation was observed beginning three weeks before the onset of the clinical symptoms. But no evidence was seen that these elevations were accompanied by a detectable systemic inflammation, as measured by quantifying C-reactive protein levels [18]. Therefore, the increased levels of cffDNA may serve as an early marker of placental damage, at a time when the maternal physiology does not yet show signs of the developing disorder.

One recently published study assessed whether levels of cffDNA, extracted from maternal plasma samples between 11 and 13 weeks of gestation from pregnant women who subsequently developed preeclampsia, were different from controls and whether the levels were related to the severity of the disease [20]. This study made use of the multicopy *DYS 14* sequence, on the Y-chromosome, as this assay is more sensitive, accurate, and efficient than that using the unique *SRY* target for the assessment of cffDNA. By targeting *DYS 14* instead of *SRY*, Zimmermann et al. demonstrated a 10-fold higher detection rate and lower quantification limit for cffDNA [21]. In the study of Sifakis et al. the median cffDNA level was higher in those patients who developed early onset preeclampsia compared to controls, whereas in the case of late onset preeclampsia, the levels were similar between cases and controls. Interestingly, cffDNA correlated significantly ($P = 0.038$) to the pulsatility index (PI) of the uterine arteries, measured by transabdominal color Doppler in patients with subsequent preeclampsia, but not in control patients. Thus the levels of cffDNA appeared to be already increased between 11 and 13 weeks of gestation in patients with subsequent early onset preeclampsia. Furthermore, these results confirmed that the levels of cffDNA were associated with the impairment in placental perfusion and with the severity of the disease.

A couple of data hints to the point that the source of cffDNA is the placenta. Initial indication came from the analysis of circulating fetal DNA in three cases of confined placental mosaicism and in a case report describing the absence of male cffDNA in a pregnancy with a male fetus but in which the trophoblast was 45,X [22,23]. More recently, Alberry et al. determined the fetal sex in plasma and chorionic tissue samples of anembryonic pregnancies [24]. In all cases, there was a concordant match for sex determination between both types of samples. Therefore, it has been suggested that the cffDNA may illustrate the underlying condition of the placenta. Indeed, the current hypothesis to explain the increased levels of cffDNA long before the onset of the clinical symptoms of preeclampsia proposes that the failure of uterine spiral arteries transformation during the early stages of placentation may induce aberrant placental perfusion [25,26]. As a consequence, the placenta may become chronically hypoxic, or most likely, alternating periods of hypoxia/re-oxygenation within the intervillous space may subsequently trigger tissue oxidative stress and may increase placental apoptosis and necrosis [27]. What likely follows is an increased shedding of necrotic and/or apoptotic subcellular syncytiotrophoblast debris that contain fetal DNA into the maternal circulation [28]. Further evidence that cffDNA may originate from the placenta via apoptosis comes from an *in vitro* study examining the effects of oxidative stress induced by 0.5% oxygen for 1 h followed by re-oxygenation, on trophoblastic tissue [29]. Tjoa et al. showed that the concentration of cell-free *beta-globin* DNA in the tissue supernatant was significantly increased 20 h after hypoxia-reoxygenation. The increased apoptosis rate of trophoblast cells was confirmed by an increased activation of the pro-apototic effector protein caspase-3. Interestingly, the authors showed that both the release of cell-free DNA and the levels of caspase-3 were significantly reduced by the addition of the antioxidant vitamins C and E into the culture medium.

In addition to the evidence for an increased release of cffDNA into the maternal circulation in preeclampsia, there is also solid prove of reduced renal clearance of these molecules in this pregnancy condition. In normal pregnancy, cffDNA is detectable as soon as 5 weeks post coitus, its levels increase with gestational age, and it completely disappear after 2 h following delivery (mean half-life for circulating fetal DNA 16.3 min, range 4–30 min) [30]. By opposition, Lau et al. demonstrated a much slower clearance rate in preeclamptic patients, with a median half-life of cffDNA clearance that was four times longer than that in unaffected women (114 min in the preeclamptic group versus 28 min in the control group) [31]. The fast disappearance rate may be due to an efficient renal clearance in normotensive women, which is impaired in preeclampsia. However, other organs, such as the liver may also contribute to the impaired clearance of circulating DNA in preeclamptic patients [32].