Review / Derleme

Cell-free fetal DNA and RNA circulating in maternal blood: review, part I

Anne kanında serbest dolaşan hücre dışı fetal DNA ve RNA: derleme, bölüm 1

Ali Çetin, MD[1]

Department of Obstetrics and Gynecology, Cumhuriyet University School of Medicine, 58140 Sivas

Abstract

This review evaluates the scientific and clinical status of the use of circulating cell-free fetal nucleic acid technology for non-invasive prenatal diagnosis, a rapidly developing and dynamic field. After the landmark discovery of cell-free fetal DNA in maternal blood during pregnancy was made more than a decade ago, it was recognized that the cell-free fetal DNA represents only a small fraction of the total cell-free DNA in the maternal circulation during pregnancy. It is known that it can be reliably detected from 5 weeks gestation and is totally cleared within a few hours of birth. The promise of that breakthrough is now being realized as the technology is translated into clinical practice for the non-invasive prenatal diagnosis.

Keywords: Cell-free fetal nucleic acids, DNA, RNA, pregnancy, non-invasive prenatal diagnosis

Özet

Bu derleme hızlı gelişen ve dinamik bir alan olan non-invaziv prenatal tanı için dolaşımda bulunan hücre dışı fetal nükleik asitlerin kullanımının bilimsel ve klinik durumunu içerir. Gebelikte anne kanında hücre dışı fetal DNA'nın bulunmasının üzerinde on yıldan fazla süre geçtikten sonra, gebelikte anne kanında bulunan serbest DNA'nın küçük bir kısmını oluşturduğu görüldü. Gebeliğin beşinci haftasından sonra ölçülebilmektedir ve doğumdan kısa süre sonra kandan kaybolmaktadır. Bu ilerlemenin sayesinde non-invaziv prenatal tanı için bu teknoloji klinik pratiğe aktarılmaya çalışılmaktadır.

[1] **Corresponding author:**
Dr. Ali Çetin, Kadın Hastalıkları ve Doğum Anabilim Dalı, Cumhuriyet Üniversitesi Tıp Fakültesi, TR-58140 Sivas
Email: dralicetin@yahoo.com

Anahtar sözcükler: Hücre dışı fetal nükleik asitler, DNA, RNA, gebelik, non-invasiv prenatal tanı

Introduction

During pregnancy, the fetal and maternal circulations are separated by the placental membrane. In contrast to popular belief that the placenta forms an impermeable barrier between mother and child; however, a variety of evidence has pointed towards the incompleteness of this barrier to cellular trafficking. There is bidirectional traffic between the fetus and the mother during pregnancy (Lo et al., 1996). Multiple studies have shown that both intact fetal cells and cell-free fetal nucleic acids (cffNA) cross the placenta and circulate in the maternal bloodstream (Wright & Burton, 2009).

Fetal Nucleated Cells

Universal presence of fetal cells in maternal blood is now accepted, but their occurrence is rare and requires complex enrichment and identification strategies (Bischoff et al., 2005). There exist one to six fetal cells per milliliter of blood from normal pregnant women (Hamada et al., 1993; Krabchi et al., 2001). Intact fetal cells circulating in maternal blood present an attractive target for NIPD, particularly for the diagnosis of fetal sex and chromosomal abnormalities by simple karyotyping. However, the difficulty in successfully detecting such fetal cells in maternal blood has been a major obstacle to the routine application of this concept. Although the existence of fetal cells in maternal blood has been known for more than a century, isolation of intact fetal nucleated red blood cells for the purpose of prenatal diagnosis was not achieved until 1990 (Bianchi et al., 1990). Since then, studies related to the fetal cells from maternal blood have been extensively investigated (Wright & Burton, 2009). Although certain fetal cells (specifically nucleated red blood cells) have a relatively short lifespan in maternal blood (Lurie & Mamet, 2000), other fetal cell types can persist in the maternal circulation for decades following pregnancy (Bianchi et al., 1996), potentially causing false-positive results in subsequent pregnancies. The largest study concerning the use of fetal cells in maternal blood was that of the multicentre National Institute of Child Health and Development (NICHD) fetal cell study group. Of 2744 maternal samples (Bianchi et al., 2002), fetal male cells were correctly identified in 41.4% when the fetus was euploid (n = 1292). Among confirmed aneuploidy cases, the detection rate was higher: 74.4%. Although further improvement of existing enrichment and isolation protocols is warranted, progress remains hampered by both rarity of fetal cells and the lack of fetal-specific cell markers (Bischoff et al., 2005).

Cell-free Fetal Nucleic Acids

Therefore, although research into sophisticated cell sorting techniques is ongoing, the majority of recent research has focused on cffNA in the maternal blood (Wright & Burton, 2009). cffNA can be detected in the maternal circulation during pregnancy form

5 weeks gestation, and they are rapidly cleared form the circulation following birth (Wright & Burton, 2009).

Nucleic acids (DNA and RNA) in plasma were first observed >50 years ago. In the early 1970s, increased quantities of DNA were verified in the plasma of cancer patients (Leon et al., 1977). Investigations into the size of these plasma DNA fragments from cancer patients has revealed that the majority show lengths in multiples of nucleosomal DNA, a characteristic of apoptotic DNA fragmentation (Giacona et al., 1998; Jahr et al., 2001).

Fetal/placental-derived RNA in Maternal Plasma

In plasma from cancer patients, RNA is also shown to be present (Kopreski et al., 1999). These molecules are likely packaged in apoptotic bodies and, hence, rendered more stable compared to 'free RNA' (Hasselmann et al., 2001). Thus, it is not surprising that stable fetal RNA is also present in maternal plasma (Poon et al., 2000). Ng et al. (2003) detected maternal plasma mRNA transcripts exclusively expressed from the placenta. Two placenta-expressed genes (human placental lactogen, hPL; b subunit of hCG) were detected in each of 10 maternal samples. This study provides direct evidence that RNA is stable in whole blood prior to processing and clearance following delivery. Investigations involving another plasma-expressed gene, corticotrophin-releasing hormone (CRH), have shown increased CRH mRNA in plasma of women with pre-eclampsia (n = 12; mean 1070 copies/ml) compared with normal gestational age-matched controls (n = 10; 102 copies/ml) (Ng et al., 2003).

Cell-free Fetal DNA

In 1997, Lo et al. (1997) were the first to show the presence of cell-free fetal DNA (cffDNA) in plasma of pregnant women. They amplified the Y chromosome-specific SRY sequence with real-time PCR in women carrying a male fetus. The origin of the cffDNA has remained uncertain, although evidence suggests that the placenta is the most likely source. Transplacental traffic of fetal hemopoietic cells is a potential alternative, but the number of these cells in the maternal circulation is considered to be too small to account for all of the cffDNA released. Clinical studies have suggested a strong association between trophoblast degeneration and the release of cffDNA into the maternal circulation (Tjoa et al., 2009). Tjoa et al. (2009) demonstrated that apoptosis related to oxidative stress induced the release of cell-free DNA from cultured placental villi.

Using quantitative real-time PCR, surprisingly high mean concentrations (6.2% of total plasma DNA) of fetal DNA were found in maternal plasma in early and late pregnancy (Lo et al., 1998b). Several reports have confirmed that gestational age correlates positively with amount of fetal DNA in plasma; thus, higher detection rates are reported with increased gestation (Bischoff et al., 2005).

Level of Cell-free Fetal DNA

As expected, detection of relatively low levels of fetal DNA sequences (as compared to maternal DNA levels) is dependent on the sensitivity of the assay as well as the amount of target fetal sequences. Little is known about the parameters governing the level of cffDNA. Several reports have confirmed that gestational age correlates positively with amount of fetal DNA in plasma; thus, higher detection rates are reported with increased gestation (Bischoff et al., 2005). Lo et al. (1998b) reported fetal concentrations to be low in the first trimester, rising in the second and third trimester. Ariga et al. (2001) combined real-time kinetic PCR with liquid oligomer hybridization with 32P-labelled probes to quantify Y chromosome-specific sequences throughout pregnancy. In 20 women confirmed to have a male fetus and followed from the first to third trimester, fetal DNA concentrations increased from 10.1 to 130.5 copies per 0.5 ml maternal plasma. Rijnders et al. (2003) studied pregnant women after assisted reproduction and reported detection of fetal DNA as early as 5 weeks and 2 days gestation in one of two patients; however, detection reached 100% by 9 weeks gestation.

During the last 8 weeks of pregnancy there is a sharp increase of fetal DNA in maternal plasma (Lo et al., 1998b). This might be related to gradual breakdown of the maternal–fetal interface/placental barrier (Bianchi, 2000). To address this, Chan et al. (2003) performed serial analysis of fetal DNA concentrations in late pregnancy, showing a positive correlation with gestational age in the third trimester. During the late third trimester, they observed a mean increase of 29.3% of fetal DNA each week. Thus, they provide normative values for comparative studies involving pregnancy related pathological conditions such as preterm labor and preeclampsia (Bischoff et al., 2005).

The clearance of cffDNA from maternal plasma after delivery appears to occur in a rapid manner. The rate of clearance will also provide information as to the applicability of cffDNA measurement in the study of the dynamic processes involved in the handling of circulating DNA during pregnancy. The notable differences between fetal cell and cell-free DNA clearance suggest that the predominant cell populations involved may be distinct. For example, it is possible that the trophoblasts may be the predominant cell population involved in the liberation of fetal DNA into the cell-free fractions. Fetal erythroblasts, on the other hand, have been postulated to be the predominant fetal cell population found in maternal blood. By the use of cell sorting and sensitive PCR assays, fetal hematopoietic progenitor cells have been shown to persist in some women, even decades after delivery. The latter phenomenon has been associated with certain autoimmune disorders.

DYS19, DYS385 and DYS392 can be used as the Y chromosome-specific STR by using the following primers:

DYS19-F5'-CTATGAGTTTCTGTTATAGT3';

R5'-ATGGCATGTAGTGAGGACA3',

DYS392-F5'-TCATTAATCTAGCTTTTAAAAACAA3';

R5' -AGACCCAGTTGATGCAATGT 3'

DYS385 - F5 '- AGCATGGGTGACAGAGCTA 3',

R5'-GGGATGCTAGGTAAAGCTG3'.

The amplification sensitivity of Y specific STR, DYS19 was 100% (22/22) in the male fetal DNA samples. The incidence of other STRs, i.e. DYS385 and DYS392 were 91% (20/22) each (Nair et al, 2007).

Use of Cell-free Fetal Nucleic Acids in Non-invasive Prenatal Diagnosis

Traditional methods of prenatal diagnosis of genetic disorders use materials obtained by amniocentesis or chorionic villus sampling, invasive procedures that carry a small but clear risk of miscarriage (ACOG Practice Bulletin No. 88, 2007). Maternal serum analyte screening and ultrasound can identify individuals at risk for fetal aneuploidy (predominantly trisomy 21), but like other non-invasive screening methods, they are hampered by non-optimal sensitivities and high false-positive (procedure) rates (Bischoff et al., 2005). The discovery of cffNA in the plasma of pregnant mothers has led to the development of several NIPD techniques in the past decade. cffDNA in the maternal blood has been shown to help predict some sex-linked diseases and fetal hemolytic disease resulting from Rh blood group incompatibility. With use of cffDNA, non-invasive prenatal diagnosis (NIPD) of several conditions has been achieved, including fetal rhesus D status (Lo et al., 1998a), myotonic dystrophy (Amicucci et al., 2000), achondroplasia (Saito et al., 2000), and certain chromosomal translocations (Chen et al., 2000; Chen et al., 2001). cffNA circulating in maternal blood can be used for the early NIPD of an increasing number of genetic conditions, both for pregnancy management and to aid reproductive decision-making (Hall et al., 2010).

However, it is important to note that in all of the above situations, investigators have been restricted to detecting genes or mutations that the fetus has inherited from the father, which are genetically distinguishable from the DNA sequences of the mother. Robust detection and quantification have been achieved when the fetal DNA sequence of interest does not have a maternal counterpart (e.g., Y chromosomal DNA, RhD gene when the mother is RhD negative) by techniques such as real-time PCR. This limitation exists because fetal DNA in maternal plasma and serum is present in an excess background of maternal DNA (Bianchi, 1998). The possible introduction of fetal DNA in maternal plasma as a routine prenatal diagnostic tool has raised questions with regard to the need of a generic marker for circulating fetal DNA (Lo et al., 1998b; Avent et al., 2000). To date, most proposals for such a marker have focused on the use of genetic polymorphisms between the mother and fetus (Pertl et al., 2000; Tang et al., 1999). Poon et al. (2002) demonstrated that differential DNA methylation between fetus and mother can be used for detecting cffDNA in maternal plasma. They suggested that the feasibility of epigenetic markers for fetal DNA detection in maternal plasma may open

up a new approach for the development of a gender- and polymorphism-independent fetal marker in maternal plasma.

The detection of fetal aneuploidy and autosomal recessive disorders with cffNA is particularly challenging because only a small portion of the cffNA in maternal plasma is derived from the fetus (Fan et al., 2010). Fan et al. (2008) demonstrated noninvasive detection of fetal aneuploidy by high-throughput shotgun sequencing of cffDNA, and their results were reproduced in two studies (Chiu et al., 2008; Chiu et al, 2010). Fan et al. (2010) demonstrated that paired-end-sequencing allows the direct measurement of the length distribution of cffDNA in maternal plasma, with single base resolution. They concluded that fetal DNA is shorter than maternal DNA and found that cffDNA had a dominant peak at approximately 162 bp and a minor peak at approximately 340 bp.

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