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Non-Invasive Prenatal Testing for Aneuploidy: A Review of the Literature

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Abstract

Aneuploidy is one of the leading causes of perinatal deaths and childhood handicaps. An important issue that has led to heated debates in the agenda of maternal-fetal medicine and that has been widely investigated in recent years is 'the isolation of fetal deoxyribonucleic acid (DNA) from maternal blood. It is agreed that fetal DNA can be used in fetal aneuploidy screening and in predicting some pregnancy complications that are considered to be of placenta-based such as preeclampsia and preterm labor. The fetal DNA testing of maternal blood is regarded as an advanced screening test and generally named as 'noninvasive prenatal test' (NIPT). In this meta-study, we will review the accumulated data on the increasing clinical use of NIPT in perinatology as well as the other presently used conventional noninvasive aneuploidy screening methods.

Keywords: Fetal DNA Non-invasive prenatal diagnosis, Trisomy 21, Trisomy 18, prenatal screening

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Introduction

Aneuploidy is one of the leading causes of perinatal deaths and childhood handicaps. In a conventional fetal aneuploidy screening today, a number of biochemical substances derived from maternal blood are assessed using ultrasonographic measurement methods and pregnant women who are at high risk for aneuploidy are identified. The presently accepted conventional antenatal screening tests primarily aim at identifying women with increased risk for frequently seen aneuploidies such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13) and Turner syndrome (45, X). Once the risky group is identified, invasive tests including amniocentesis (AS), chorionic villus sampling (CVS) and cordocentesis are administered in an attempt to obtain the fetal genetic material for a definitive diagnosis. However, invasive tests are expensive and may involve 1% pregnancy loss rate [1]. An important issue that has led to heated debates in the agenda of maternal-fetal medicine and that has been widely investigated in recent years is 'the isolation of fetal deoxyribonucleic acid (DNA) from maternal blood'. It is agreed that fetal DNA can be used in fetal aneuploidy screening and in predicting some pregnancy complications that are considered to be of placenta-based such as preeclampsia and preterm labor. The fetal DNA testing of maternal blood is regarded as an advanced **screening test** and generally named as **'noninvasive prenatal test' (NIPT)** [2-4].

In this meta-study, we will review the accumulated data on the increasing clinical use of NIPT in perinatology as well as the other presently used conventional noninvasive aneuploidy screening methods.

Aneuploidy Screening

Every woman has the risk of giving birth to an aneuploid child. Therefore, all pregnant women should be advised to go through a fetal aneuploidy screening routinely regardless of their age and risk status in their previous pregnancies [5].

Maternal Age:

While the risk of aneuploidy was used to be calculated using only the maternal age in 1970s, the maternal serum analytes and detailed ultrasonographic examination were introduced to screening in 1980s. Many aneuploidies increase with maternal age. The risk of Trisomy 21 is

1/1000, the risk of Trisomy 18 is 1/2500 and the risk of Trisomy 13 is 1/8000 at Gestational week 12 in a women aged 20 years, whereas such risks rise up to 1/250, 1/600 and 1/1800 respectively in a women aged 35 years. Trisomy 21 can be determined at a rate of 30% (with 5% false positivity) by using maternal age only. The prevalence of Turner syndrome and other sex chromosome anomalies are not affected by maternal age [1].

Maternal Serum Biochemistry

In an aneuploidy screening, various products are used such as Alpha Fetoprotein (AFP), free beta human Chorionic Gonadotrophin (β-hCG), Inhibin A, Pregnancy-Associated Plasma Protein A (PAPP-A), and unconjugated Estriol (uE3), which are derived from the foetoplasental unit and are assessed in maternal blood. The average maternal serum levels of pregnant women having a healthy fetus are measured and the results are itemized as multiples of median (MoM). The parameters in maternal serum biochemistry are combined to each other and are used for screening purposes. These screening tests are named as double test (free β-hCG and PAPP-A) in the 1st trimester and as triple test (AFP, free β-hCG and uE3) and quadruple test (AFP, uE3, free β-hCG and Inhibin A) in the 2nd trimester [1, 6, 7].

It has been found that free β-hCG increases (2 MoM on the average) and PAPP-A decreases (0.5 MoM on the average) in Trisomy 21 in the 1st trimester, whereas both of them decrease in Trisomy 18 and 13 [8, 9]. In sex chromosome anomalies, on the other hand, free β-hCG is normal and PAPP-A is decreased [10].

Fetal Nuchal Translucency

In 1992, Nicolaides and associates proposed that the fetal nuchal translucency (NT) be measured using ultrasonography during Trisomy 21 screenings [11]. The fetal NT measurement has been shown to be an effective method in searching for Trisomy 21 and other major aneuploidies in many studies carried out thereafter [12-14]. A NT measurement should be performed by persons who received the appropriate training [15]. As the fetus grows, the NT values also increase under normal conditions. NT can be measured in an abdominal or vaginal way; ideally between weeks 11 and 13 + 6. The crown-rump length (CRL) in these weeks is between 45 and 84 mm. The median CRL-independent NT has been found to be 2

mm in euploid groups and 3.4 mm in Trisomy 21, 5.5 mm in Trisomy 18, 4.0 mm in Trisomy 13 and 7.8 mm in Turner syndrome [14,16].

An assessment of NT in combination with maternal age, free β-hCG and PAPP-A is called a combined test and it can detect Trisomy 21 at a rate of 85-95% in the first trimester with a 5% false positivity rate [1].

Other Ultrasonographic Markers used in First Trimester Aneuploidy Screening

Absence of nasal bone, reversed a-wave in a Doppler assessment of ductus venosus and tricuspid regurgitation is quite sensitive and specific like NT in a Trisomy 21 screening. When they are assessed together with a combined test, the rate of diagnosis becomes 93-96% with a 2.5% false positivity rate [17, 18]. In Trisomy 13, fetal heart rate is found to increase in the first trimester [19].

The effect of adding fetal heart rate to the combined ultrasound and maternal biochemical profile on Trisomy 21 and 18 detection is minimal in the first trimester. However, its effect on the detection of Trisomy 13 is quite good and raises the diagnosis rate from 72% to 87% [20].

Aneuploidy Screening in Multiple Pregnancies

The prevalence of multiple pregnancies increases from day to day due to more frequent use of assisted reproductive techniques (ART) and increased pregnancies at advanced ages. The widespread use of ART increases both the number of rarely seen triplet and quadruplet pregnancies in addition to twins and the rate of prematurity and congenital anomalies [21-23].

In twin pregnancies, a chromosomal anomaly screening is performed through a combined assessment of maternal age and fetal NT thickness [24].

To increase the performance of an anomaly screening, maternal serum biochemical analytes can also be used (after appropriate arrangements are made according to chorionicity). In dichorionic twin pregnancies, maternal serum β-hCG and PAPP-A are increased twice as much as those of singleton pregnancies in gestational weeks 11 + 0–13 + 6. However, the levels are less in monochorionic twins than in dichorionic twins [25,26].

While the normal value of free β-hCG and PAPP-A is approximately 1.5 MoM at weeks 8-9 in dichorionic twins, it becomes 2.0 MoM at weeks 13-14. The normal value of free β-hCG and PAPP-A is close to that of singleton pregnancy at weeks 8-9 in monochorionic twins. At weeks 13-14, free β-hCG becomes 2.0 MoM and PAPP-A 1.5 MoM [7,26]. When calculating Trisomy 21 risk in dichorionic twins, each fetus is assessed separately; fetal NTs are measured, they are assessed together with maternal age and a separate risk is established for each fetus. The rate of detecting Trisomy 21 in this way is 75-80%. The rate of false positivity is 5% for each fetus and 10% for the whole pregnancy [24,27].

Fetal NT thickness is measured separately for each fetus in monochorionic twins and then the average of the two values is taken. The rate of false positivity in a fetal NT screening is higher in monochorionic twin pregnancies than in dichorionic ones. Increased NT chromosomal anomalies in monochorionic twins may indicate cardiac defects or various fetal malformations as well as an early manifestation of twin-to-twin transfusion syndrome [28].

The PAPP-A MoM value decreases and free β-hCG MoM value increases in twins involving a fetus with fetal Trisomy 21 as in singleton pregnancies [7].

cf-DNA in Maternal Serum

As well known, placenta is a tissue with two-way permeability (from mother to fetus and from fetus to mother) and there is a heavy traffic between the fetal and maternal sections during the entire pregnancy [28]. Research has been carried out for many years to examine the fetal genetic structure using a noninvasive method. In 1997, Lo et al. showed for the first time the DNA of a male fetus in the serum of a pregnant woman [29]. Following this, studies for isolating and proliferating fetal cells in maternal circulation to use them in prenatal screenings have gained great speed.

Origin and Characteristics of Free Fetal DNA in Maternal Circulation

Free DNA in maternal circulation is named as cell-free (cf) as it does not have any cell membrane. Cf-DNAs are tiny (< 200 base pairs) DNA pieces flowing freely in plasma. There are cf-DNAs that belong to both the mother and the fetus in maternal circulation, but a large part of these (%85-90) belong to the mother. Studies exploring the origin of fetal cf-DNAs

have shown that they originate from placenta to a larger extent and from fetal hematopoietic cells to a lesser extent [30].

Fetal cf-DNAs in maternal blood can be detected from weeks 4-5 of pregnancy and their concentration increases as the pregnancy advances. Their half-life is rather short and rapidly declines to the levels that cannot be identified 2 hours after the birth [31]. For this reason, previous pregnancies in multigravida women are thought to have no effect on the cf-DNA test [31,32].

The potential mechanisms that play a role in the elimination of fetal cf-DNA in circulation include maternal plasma nuclease, hepatic cleansing, renal cleansing and fetal DNA destruction as a result of interaction with maternal cells [32-34].

Fetal Fraction:

The proportion of fetal cf-DNA to total DNA is called fetal fraction (FF) (FF= fetal cf-DNA / maternal cf-DNA + fetal cf-DNA). If the percentage of fetal fraction is less than 3-4%, it is not suitable for analysis [35,36]. The failure rate of a test due to low FF has been reported to be between 0.9 and 8.1% [35-37]. The average fetal fraction at weeks 11-13 of pregnancy is 10%. Fetal fraction decreases as maternal weight increases and the reason for this are thought to be the dilution associated with increased maternal volume. Therefore, a cf-DNA aneuploidy screening may not be the best screening method in obese and morbid obese women [36].

As free β-hCG and PAPP-A increase in maternal serum, fetal fraction also increases. These changes are argued to be the increases or decreases related to the change in placenta size. The value of fetal fraction has been shown to not be affected by sex, maternal age, race, time of storing the serum sample, or NT thickness [37]. It has been reported that the reason for the rare discrepancies in cf-DNA results may be due to maternal malignity [38].

Fetal fraction has been found to be similar to those in euploid pregnancies in Trisomy 21 and 13, but noticeably decreased in Trisomy 18. It is known that the β-hCG and PAPP-A values are low and the development of placenta and fetus is retarded in Trisomy 18. For this reason, this decrease in fetal fraction has been linked to the smallness of placenta. It was shown in a multivariate analysis that was conducted later that Trisomy 18 did not have an independent

effect on fetal fraction. It was concluded then that fetal karyotype did not affect fetal fraction and thus it could be used in both high-risk and low-risk pregnancies [38, 39].

Information on fetal and maternal cf-DNA is summarized in **Table 1**.

Table 1. Characteristics of maternal and fetal cell free-DNA

Characteristics	***Maternal cf-DNA***	***Fetal cf-DNA***
Origin	Hematopoietic	Placenta
Kinetics	Concentration increases as pregnancy weeks progress	Concentration increases as pregnancy weeks progress Rapidly cleared from maternal circulation after birth
Fraction in maternal blood	90%	10%
Size	Short DNA pieces <200 bp) Larger than fetal DNA	Short DNA pieces <200 bp) Smaller than maternal DNA

bp = base pair (unit used for DNA length) (1 bp = 340 picometer)

Fetal DNA Freeing Mechanisms

The mechanisms relating to the release of fetal cf-DNA into maternal circulation can reportedly include necrosis and/or apoptosis of fetal/placental cells, and active secretion or terminal differentiation of fetal DNA, apoptosis being the most probable candidate [40].

Apoptosis is characterized by breaking of DNA to pieces and it is not a random but a controlled process. As a result of apoptosis, DNA breaks to pieces of various sizes. Studies have shown that fetal cells commit controlled active apoptosis [41,42].

Clinical Uses of cf-DNA:

Determination of Fetal Sex:

The first use of noninvasive prenatal test was to determine fetal sex. Lo et al. identified sequences of Y chromosome in the serum of a woman carrying a male fetus using the conventional Polymerase Chain Reaction (PCR) technique [29]. In many studies that followed this one, sequences of Y chromosome such as Single Copy Sex Determining Region Y (SRY)

and Y Chromosome Specific Sequence (DYS14) were analyzed using real-time PCR. The overall sensitivity of the test was 95.4% and specificity 98.6% in determining male fetuses [43].

Conventionally, fetal sex can be determined at around week 13 with ultrasound and from week 6 when cf-DNA is employed. The identification of fetal sex in early weeks is important for the management of some diseases. For example, if the sex of a fetus with congenital adrenal hyperplasia is found to be female, maternal steroid treatment is applied; if male, then this treatment is not given. Identification of sex from maternal blood is also useful for X-related congenital diseases such as Duchene muscular dystrophy and hemophilia [39,44].

Detection of Single Gene Disorders:

An analysis of free fetal DNA makes it possible to detect inherited diseases including achondroplasia, hemoglobinopathy, congenital adrenal hyperplasia, cystic fibrosis, Huntington disease and myotonic dystrophy [45].

Fetal Rhesus D Genotyping:

In Rhesus D negative (RhD -) pregnancies, cf-DNA in maternal plasma can be used to determine the fetal RhD status. This can prevent pregnant women having an RhD (-) fetus from taking anti-D immunoglobulin unnecessarily. In a meta-analysis of the large-scale studies, it was found that the sensitivity of the test in determining the fetal RhD status was 99.5-99.8% and its specificity 94.0-99.5% [46].

Prediction of Gestational Complications:

Studies have been conducted to explore the predictive and diagnostic role of cf-DNA in some placental pregnancy complications such as preeclampsia, preterm delivery, spontaneous abort and IUGR. It has been suggested that placental lesions cause increased cell traffic in the maternal peripheral tissue before the onset of disease symptoms. The cf-DNA levels have been examined in preeclampsia-complicated pregnancies and shown to be elevated in early pregnancy before the emergence of the disease [47-49]. However, another more recent study has reported that a cf-DNA measurement does not predict preeclampsia between weeks 11-13

and weeks 20-24 [50]. Information on the use of cf-DNA in predicting preterm birth is controversial.

Farina et al. have shown that the cf-DNA levels are elevated in maternal serum in high-risk gestational diseases involving early membrane rupture or preterm birth [51]. Illanes et al. were not able to show a significant relationship between the maternal cf-DNA levels and the gestational week at the time of birth, which supports the hypothesis that cf-DNA levels are incapable of predicting early birth in women with a short cervix [52]. Quezada et al. have evidenced that a pregnancy at weeks 11-13 cannot predict a spontaneous preterm birth [53].

Aneuploidies:

The cf-DNA in maternal blood has been used in recent years in detecting Trisomy 21, 13 and 18 as well as sex chromosome anomalies and triploidy. The most frequently used methods in measuring cf-DNA include the Massively Parallel Shotgun Sequencing (MPSS) analysis, Chromosome-Selective Sequence (CSS) analysis and Single Nucleotide Polymorphism (SNP) analysis. In MPSS, maternal and fetal cf-DNAs are sequenced separately for each chromosome. Then, the relevant chromosome (e.g. chromosome 21) is quantitatively numbered and compared to the results of normal pregnancies [54]. In CSS, cf-DNA is explored for only the chromosomes in concern (13, 18, 21, X and Y) [55]. In an SNP analysis, the presence of single nucleotide polymorphism inherited from the father is explored in maternal plasma [56].

Based on the results of a large number of studies, it is accepted today that the cf-DNA analysis of the maternal blood can be used safely in singleton pregnancies when doing a fetal trisomy screening. Gil et al. [57] have reported based on their detailed meta-analysis that a Trisomy 21 screening using cf-DNA in singleton pregnancies is superior to the combination of all other screening methods (mother's age, first and second trimester ultrasound results and biochemical analysis of the first or second trimester serum). However, the performance of the cf-DNA screening test for Trisomy 18 and 13 syndromes was not found better than a combined test.

The results of the meta-analysis carried out by Gil et al. are summarized in **Table 2** [57].

Table 2. Rates at which a cf-DNA analysis can detect certain aneuploidies in singleton pregnancies.

Aneuploidy Name	**Total Detection Rate (%, 95% CI)**	**Rate of false positivity (%, 95% CI)**
Trisomy 21	99.2 (98.5–99.6)	0.09 (0.05–0.14)
Trisomy 18	96.3 (94.3–97.9)	0.12 (0.07–0.20)
Trisomy 13	91.0 (85.0–95.6)	0.13 (0.05–0.26)
Monosomy X	90.3 (85.8–94.1)	0.23 (0.14–0.34)
47,XXX; 47,XXY; 47,XYY	93.0 (85.8–97.8)	0.14 (0.06–0.24)

Use of NIPT in Multiple Pregnancies

Cf-DNA analysis is rather complicated in twin pregnancies. In monochorionic twins, since the twins with the same genetic structure (almost always both fetuses are affected or normal at the same time) give the same alleles of cf-DNA to maternal circulation, the aneuploidy risk assessment is similar to that of singleton pregnancies. The majority of dichorionic pregnancies, on the other hand, are dizygotic. The amount of cf-DNA given by each fetus to maternal circulation may differ [58].

In their study using the fetal DNA selective sequence analysis, Struble et al. aimed at measuring the fetal fraction in twin pregnancies. The two fetuses give the same cf-DNA alleles to maternal circulation in monochorionic twins; thus, their fractions are similar to singleton pregnancies [59]. Three previous studies made on twin pregnancies have shown that each fetus has adequate cf-DNA contribution to maternal circulation [60-62]. However, in dizygotic twins, the contribution of each fetus to the DNA pool in maternal circulation is different. Therefore, there may be confusion in diagnoses and a decline in the success of the test [63]. In a recent meta-analysis made by Gil et al., the rate of detecting Trisomy 21 for twin pregnancies was found as 93.7% (95% CI, 83.6-99.2%) and the rate of false positivity as 0.23% (95% CI, 0.00-0.92%) [57].

In conclusion, when all the studies are reviewed, it seems that combining the results of the first trimester ultrasound and biochemical results of a Trisomy 21, 18 and 13 screening with the results of a cf-DNA analysis of maternal blood will improve the final screening results. However, it should be noted that the cf-DNA analysis is still defined as a screening method.

With cf-DNA, false positive and false negative results may be obtained. A negative cf-DNA result indicates a low risk and does not rule out Trisomy 21 and other chromosomal conditions completely. For this reason, CVS or amniocentesis should be recommended for women with abnormal cf-DNA results or those who require definite information on the chromosome status of a pregnancy [64].

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Conflict of interest

None of the authors has any conflict of interest.

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