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Comparison of traditional prenatal diagnosis procedures and Cell-Free DNA in maternal plasma as a new molecular approach for prenatal diagnosis

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Abstract

Cell-free DNA (cfDNA) is a moderately novel method with an exponential expansion in uses for a wide range of applications. The clinical services of these new techniques will be estimated by the scientist in the field via the newest case studies. Non-invasive detection of cell-free DNA has the potential to impact treatment regimens and clinical protocols. cfDNA will manage the standard of care in transplant medicine, oncology, and cardiovascular disease. This field is appearing as one of the most exciting and promising areas of medicine and other sciences and can make a huge influence on prenatal care. The aim of this review is to present potential and application of Cell-Free DNA in prenatal diagnosis.

***Keywords*:** Cardiovascular disease, Cell-Free DNA, Prenatal diagnosis, Non-invasive

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Introduction

One of the major factors limiting the survival of some patients may be the late prenatal diagnosis. Instrumental techniques are unable to detect prenatal diseases and cancer in situ; but, as prenatal or cancer-related molecules, including cell-free DNA (cfDNA), from a fetus or tumor, may enter the blood circulating(in prenatal disease enter the maternal blood circulating) through the affected organs, it is probable that these molecules can be detectable in samples of systemic blood(1). The cfDNA concentration has been investigated in patients with various types of cancer or in the serum of maternal for prenatal diagnosis (2, 3). In cancer detection situation, cfDNA is often practiced as a DNA source to discover the loss of heterozygosity(4), promoter methylation (5, 6)and cancer cell-derived mutations(6). Increased levels of cfDNA are shown not only in patients with tumors but also in individuals with premalignant lesions, trauma or inflammation(7, 8). The cfDNA in plasma and serum has been calculated by many techniques, including quantitative PCR (qPCR)(9), the PicoGreen assay (10, 11) and

spectrophotometry(12, 13). So, the employment of cfDNA-based diagnosis and prognosis in the laboratory and clinic is a challenge because of the trouble in normalizing and comparing the present data, the relatively small number of samples studied in some cases and the lack of technical standardization. In this review, we aimed to present the application and extraction methods of cfDNA in human blood using different methods.

Prenatal diagnosis: conventional and current techniques:

There are a variety of prenatal screening tests to help identify if a pregnant woman is at an increased risk of carrying a baby with birth defects. There are three kinds of screening tests. Testing can start as early as 10 weeks, and involves one or two blood tests and possibly an ultrasound depending on the certain testing technique chosen. Prenatal diagnosis Methods can be categorized into two parts: new methods and conventional techniques

Conventional Techniques:

Ultrasound:

The test is used to determine the position and sizeof the fetus, as well as any potential abnormalities in the growing bones and organs of the baby(14). A special ultrasound called a nuchal translucency ultrasound is done between 11 weeks and 2 days and 14 weeks and 2 days. Nuchal translucency refers to the accumulation of fluid at the back of the baby's neck(14). Nuchal translucency may be increased in cases of Down syndrome and various types of birth defects, especially in heart defects. It is used to carefully evaluate the baby from head to toe for any birth defects, although not all birth defects are visible by ultrasound. A level 2 ultrasound is performed if a level 1 ultrasound is abnormal, or if the prenatal screening tests are abnormal. (15)

Blood Test:

Pregnancy woman should have a blood test to ensure she isimmunized against rubella and to screen for three infections(16): syphilis, hepatitis B, and HIV. A blood test will also be used to determine the mother's blood type and Rh factor. The blood test will be used to specify the pregnant woman's Rh compatibility with her growing fetus. (17)

Prenatal Screening Tests:

There are three kinds of blood tests that are suggested as part of prenatal screening for birth defects. The blood tests searched at proteins produced by the baby and measure the levels of these proteins in the mother's blood. There are a total of six fetal proteins that can be tested. These tests determine babies with Down syndrome and other genetic conditions such as Trisomy 18 (18).

Prenatal Diagnostic Tests (PND):

Diagnostic tests evaluate individual pregnancies and leave virtually no doubt asto the existence or absence of birth defects. In table 1 is revealed some invasive and non-invasive techniques.

These methods are more invasive than screening tests and are often only performed if there is a specific concern if a screening test shows a problem, or if age, family history, or medical history indicates an increased risk for a problematic pregnancy(19).

Amniocentesis:

During amniocentesis, amniotic fluid is taken from the uterus for testing. Amniotic fluid around the baby during pregnancy; it contains fetal cells with the same genetic makeup as the baby, in addition todifferent chemicals produced by the baby's body. This diagnostic test is used in a number of methods.(20)

Genetic Amniocentesis:

Genetic amniocentesis evaluates genetic abnormalities like Down syndrome and spina bifida. (21)Genetic amniocentesis is usually performed after week 15 of the pregnancy and may be considered if(22):

- ✓ A prenatal screening test indicated abnormal results.

- ✓ You had a chromosomal abnormality during a past pregnancy.
- ✓ The age is 35 or older.
- ✓ Whenthere is a family history of a certain genetic disorder.

Table1: Invasive and non-invasive techniques features

Method	Description	usually performed time	reference
Invasive techniques			
Amniocentesis	Amniocentesis means the extraction amniotic fluid from the abdominal wall to investigate embryonic chromosomal abnormalities	15 - 20 weeks gestation	(23, 24)
Biopsy of fetal tissue	In the utero samplingof the liver, skin, and muscle of the fetus to investigate embolic disorders	Around 20 weeks gestation	(25, 26)
Fetal blood sampling	also known as cordocentesis or percutaneous umbilical cord blood sampling	17-18 weeks gestation	(27, 28)
Chorion Biopsy	a sample of chorionic villi	10-11 weeks gestation	(29, 30)
NON-Invasive techniques			
Ultrasound	Ultrasound is used in the following conditions : assessment of fetal viability, confirmation of gestational age, the establishment of chorionicity in multiple gestations	18 and 20 weeks gestation	(31)
Cell-free fetalDNA	Cell-free fetal DNA (cffDNA) is fetal DNA which can circulate freely in mother's bloodstream- The origin of apoptotic trophoblastic placental cells, other fetal cells	after 5 to 7 weeks gestation	(32, 33)

Maturity Amniocentesis:

Maturity amniocentesis is performed to identify whether or not a baby's lungs are ready for birth. This diagnostic test is only done if a planned early delivery (either an induction of labor or a cesarean) is being considered for medical reasons. It is typically done between weeks 32 and 39.(34)

Other Types of Amniocentesis:

Although all of these types of amniocentesis are very rare, the procedure may also be performed to check a baby for infection, to reduce the volume of amniotic fluid, to diagnose a uterine infection, or to evaluate the seriousness ofan Rh incompatibility among mother and fetus (35).

Chorionic Villus Sampling:

Chorionic villus sampling (CVS) is a prenatal test used to check for geneticdisease in the fetus likeTay-Sachs disease, cystic fibrosis, and chromosomal abnormalities such as Down syndrome(36). A CVS will not show all birth defects. During CVS, a sample of chorionic villi is taken from the placenta and is then utilized to test various parts of the fetus. The chorionic

villi make up most of the placenta and share the baby's genetic makeup. A CVS test is typically done during weeks 10 to 12(37).

New strategies for PND:

The advantages of new methods forPND are rapid aneuploidy detection, reliable and cost-effective for detecting the targeted fetal aneuploidies, but are limited in their ability to detect non-aneuploidy chromosome abnormalities, some of which are clinically significant(38). Currently available techniques for PND include fluorescence in situ hybridization (FISH) and quantitative fluorescence polymerase chain reaction (QF-PCR)(39). Multiplexligation-dependent probe amplification (MLPA) is a newermethod under investigation. FISH and QF-PCR are known to, and MLPA is proving to, haveas same as sensitivity and specificity to full cytogenetic karyotyping to detect of fetal aneuploidy (for chromosomes 13, 18, and 21 and the sex chromosomes). Advantages of QF-PCR and MLPA over full karyotype includesubstantially decreased turnaround time and automation andbatching of samples leading to reduced cost for persample(40). Other methods that are using in PND are Digital PCR, Nanomagnetic method and Microarray.In the future, all of them may be a suitable tool for prenatal diagnosis for a subset of women undergoing invasive testing only for an increased risk of fetal aneuploidy chromosomal rearrangement such as a balanced translocation(۴۱).

Fluorescence in Situ Hybridization Technique:

FISH uses a fluorescently labeled probe to target a unique sequence of DNA that it selectivelybinds. For prenatal samples, FISH is performed on uncultured,interphase cells(42). The probes arespecific for 5 chromosomes including 13, 18, 21, X, and Y(43).

FISH is can also detect several common chromosomal microdeletions associated with structural fetal abnormalities, and it was found to be virtually 100% sensitive and 100% specific for the detection of the targeted aneuploidies. Another advantage is that it can detection of triploidy(43). The biggest limitation of this technique is its unsuitability for automation.

Quantitative Fluorescence Polymerase Chain Reaction Technique:

QF-PCR is a well-established molecular geneticmethod that selectively amplifies the specific site of genomicDNA based on the binding of primers that areunique to that site(44).On DNA, QF-PCR amplifies specific polymorphicDNA hallmarks for the specific chromosomes such as 13, 18, 21, X and Y). Fluorescentlylabeled primers attach to perspecific sequence and allowDNA polymerase to replicate DNA(45).

QF-PCR is as reliablewith sensitivity and specificity of 95.65% and 99.97%, respectively. It determines mosaicism at a level similar to that of full karyotype. The main advantage over FISH is amenability to automation, which decreases the cost persample(46).

Multiplex Ligation-Dependent Probe Amplification Technique:

MLPA is a new PCR-based method thatdiscriminates between copy numbers of specific sequencesof DNA(47). MLPA uses two-part probes of unique lengththat, when hybridized to adjacent target sequences ongenomic DNA, can be joined together by the enzymeDNAligase. This then allows alltarget sites to be amplified using asingle primer pair that is complementary to the two freeends common to all probes(48).

MLPA panels are also beingdeveloped to screen for dozens of such conditions (such as Prader-Willi and Angelman)that would otherwise not bedetected prenatally. This way is proving to be arapid, simple, and reliable technique with the cost comparable toQF-PCR(49). The sensitivity of 100% and specificity of 99.8% fornon-mosaic targeted aneuploidies have been reported. It alsosuggestsmanyadvantages, including low cost and the abilityto amplify multiple markers in one tube. Disadvantagesinclude the inability to determine all cases of triploidy and unknown sensitivity for mosaicism(50).

Digital- PCR:

This technique has been planned to let the detection of a variety of genetic phenomena, including the recognition of the loss of heterozygosity (LOH) in tumor samples and plasma of cancer patients.(51)This technique is precisefor measurement of cell-free fetal DNA in maternal plasma and it facilitates noninvasive prenatal diagnosis of fetal chromosomal aneuploidies and other applications. Computer imitation and practicalproof confirmedaccurateness of the disease classification algorithm(52).Digital PCR involves multiple PCR analyses or extremely dilute nucleic acids such that most positive amplifications reflect the signal from a single template molecule, permitting the counting of individual pattern molecules. The proportion of positive amplifications among the total number of PCRs analyzed allows an estimation of the template concentration in the original nondiluted sample. Because ofpattern molecule quantification by digital PCR does not depend on dose–answerassociations between reporter dyes and nucleic acid concentrations, its methodical precision should supposedly be superior to that of real-time PCR. (53)

Microarray:

Chromosomal microarray analysis is a method that can recognize major chromosomal aneuploidy in addition to submicroscopic abnormalities that are too small to be identified by conventional karyotyping.Microarray analysis also can give information at the submicroscopic level in the whole of the human genome. These submicroscopic rearrangements may responsible for a sizable portion of the human genetic disease burden, with some estimates as high as 15% (54).

Two kinds of microarrays use in clinical prenatal testing: comparative genomic hybridization (CGH) and SNP arrays. Although both of these methods identify copy number variants, they detectmany types of genetic variation. Chromosomal microarray analysis does not need dividing cells, it may be useful in the evaluation of fetal death or abortion, in which the culturing of macerated tissue is frequently failed(55). In addition, chromosomal microarray analysis is a standardized way that involves the use of computerized analysis.Chromosomal microarray analysis is oftenused when ultrasonographic examination detects fetal structural anomalies(55). Although traditional karyotyping, chromosomal microarray analysis cannot identify balanced inversions, balanced translocations, or all samples of tissue mosaicism. Furthermore, not all microarrays can identify triploidy, even though most triploid fetuses can be identified by ultrasonography(56).

Nanomagnetic:

Nanodiagnostics involve the use of nanotechnology in clinical diagnosis to meet the demands for increased sensitivity and early detection in less time. The large surface area of nanomaterial enables attachment of a large number of target-specific molecules of interest for ultra-sensitive detection.Magnetic nanoparticles have been demonstrated to have exciting and promising applications in medical diagnostics and therapy as well as immunoassay based diagnostics(57).

Magnetics can help by providing detectors and actuators that are naturallyrough, small, and physique-manufacturable.

Magnetic nanoparticle (MNP) -based gene transfection has been shown to be an effective, non-viral technique for delivery into cells in culture. These advantages have been demonstrated in a number of primary cells and cell lines.Reported that oscillating magnet array-based nanomagnetic transfection significantly improves transfection efficiency in both human prenatal cardiac progenitor cells and adult cardiomyocytes when compared to static magnetofection, electroporation, and cationic lipid components, while maintaining high cell viability. This technique demonstrated*in vitro* delivery of the reporter plasmid pEGFP-N1 using the oscillating magnet array and MNP in human prenatal cardiac progenitor cells and adult cardiomyocytes and compare it to other non-viral transfection techniques(58).

Cell-free fetal DNA:

any scientist and genetic societies have offered NIPT using Massively Parallel Sequencing (MPS) of maternal plasma cffDNA testing to women at high risk for prenatal(59).

Cell-free fetal DNA (cffDNA) is fetal DNA which can circulate freely in the maternal bloodstream and can be easily sampled by venipuncture on the mother. Investigation of cffDNA gives a non-invasive technique of prenatal diagnosis. cffDNA originates from the trophoblasts(60, 61) and It is estimated that 2-6% of the DNA in the maternal blood have fetal originality(61). The fetal DNA is fragmented by apoptosis (programmed enzymatic activity) which is frequent in healthy peoples or necrosis, which is frequent in patients. By this fragmentation process, make possible the way into the maternal bloodstream via shedding of the placental particles into the maternal bloodstream(62). It's have shown that cffDNA can primary be detected as early as 7 weeks gestation and the quantity of cffDNA can be proliferated as the progress of pregnancy(63)and quickly diminishes after the birth of the baby and approximately 2 hours after birth it is no longer detectable in the maternal blood(64). The size of cffDNA is significantly smaller than DNA of the maternalorigin in the bloodstream, by approximately 200bp fragments in size(65).Figure 1 explains the difference between amniocentesis and cfDNA diagnosis applications.

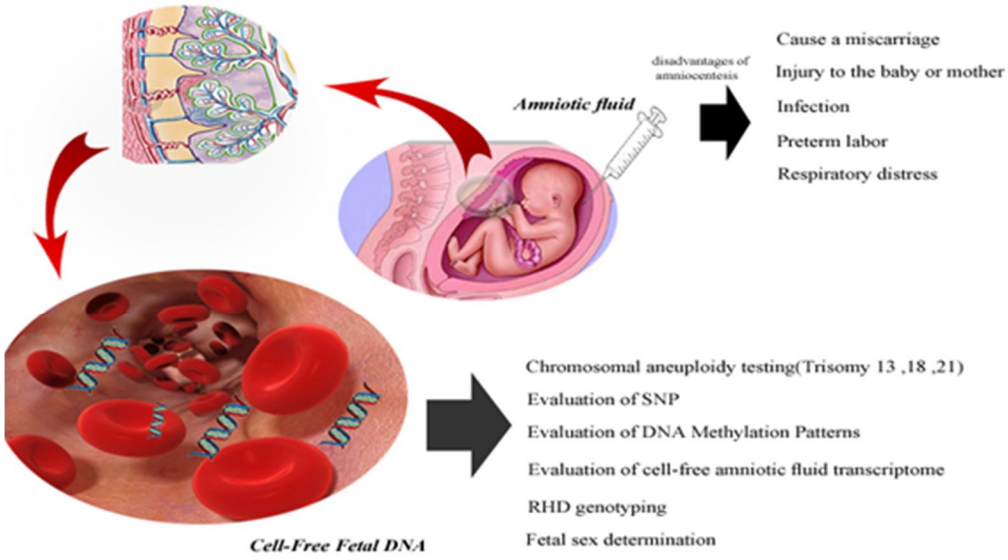

Fig1: Explaining thedisadvantages oftraditional prenatal diagnosis procedures and clinical applications of Cell-Free DNA in maternal plasma as a new molecular approach for prenatal diagnosis.

Extraction methods of cfDNA:

This DNA, existent just in tiny concentrations in serum or plasma, various techniques have been applied to purify CFDNA, including using chromatography resins(66, 67), modified salting-out(66), guanidiumthiocyanate(68) or magnetic beads(69), but The lack of consensus regarding which extraction method is better for the efficient capture of such DNA are remains.

In this method, Siew Lee Fong et.al evaluated in parallel 7 extraction approaches (Phenol-chloroform method with the addition of glycogen, Sodium iodide method, Guanidine-resin method, QIAamp DNA Blood Midi kit with carrier RNA, Charge Switch 1-mL serum kit, ZR serum DNA, Puregene DNA purification System And Cell and Tissue Kit). The phenol-chloroform procedure (PCI-glycogen), QIAamp DNA blood kit and sodium iodide method (NaI method) generated

significantly higher recoveries of DNA, evaluated by fluorescent measurement, than the other 4 approaches. Measured by TaqMan real-time PCR (RT-PCR) technique targeting on CDH1 (amplicon size: 68 bp), it has shown the NaI approach was rated top between all approaches. Moreover, the higher isolation of DNA obtained with the NaI and PCI glycogen methods were also shown on the agarose gel. In comparing the NaI approaches and the PCI-glycogen method, they showed that the latter was not only superior to the former in terms of DNA quantity, as evaluated by 2 rounds of PCR, but also was less costly, more rapid, and simpler. The results of the study, have shown the NaI method reliably revealed better performance(70).

Clinical Applications of Cell-Free Fetal DNA from Maternal Plasma:

It can be detected in maternal serum as early as the 7th week of gestation and that it then increases in concentration as pregnancy progress(71). The use of cell-free DNA that circulated in maternal serumhas attractedthe attention of clinicians in oncology, prenataldiagnosis, and hematologyrecently and may be a valuable source of material for noninvasive prenatal diagnosis.Several clinical applications have been described such as(71):

- ✓ Sex determination (using PCR targeted at the SRY gene)
- ✓ Fetal rhesus-Dstatus
- ✓ Prenatal DNA diagnosis of a single-gene disorder
- ✓ Prenatal diagnosis of X-linked disorders

Sex determination (using PCR targeted at the SRY gene):

In X-linked conditions,primary and reliable noninvasive the fetal sex determination by observing cfDNA in the maternal bloodstreammay eliminate the need for invasive testing(72). Other techniques for female sex determination before the using cfDNA are invasive and carry out at 11 weeks of gestation. In other hands, cfDNA can be observed in the maternal circulation blood at 5 weeks of gestation and clears within several hours after birth(73). There is a minor risk of abortion(74). The main manner is targeting the SRY gene on the Y chromosome and DYS14 sequence(75).

Fetal rhesus-D status:

A noninvasive test for fetal Rh(D) cfDNA status in the maternal blood circulation is commercially accessible widely used in the United States and Europe to decrease the need for unnecessary fetal surveillance services in isoimmunized Rh(D)-negative women gestating an Rh (D)-negative fetus. Nevertheless, as the specificity and sensitivity of the test are not 100% accurate.Rhesus blood group (D antigen) is always applied to assess the risk of hemolytic disease in the fetus. In this disease, the maternal antibodies destroy RhD-positive fetal red blood cells. These situations cause lethality for the fetus.

Prenatal DNA diagnosis of a single-gene disorder:

Prenatal DNA diagnosis is usually done via invasive procedures such as amniocentesis and chorionic villus sampling. Fetal nucleated erythrocytes in maternal blood have been proposed as potential target cells for non-invasive prenatal diagnosis. Maternal blood is an unequal mixture of small (and fragmented) amounts of fetal DNA within a wide background of maternal DNA.

The use of cell-free fetal DNA in maternal plasma for the diagnosis of single-gene disorders is limited to disorders caused by a paternally inherited gene or a mutation that can be distinguished from the maternally inherited counterpart.

Prenatal diagnosis of X-linked disorders:

The gold standard for prenatal diagnosis of genetic disorders is still an invasive method. cfDNA circulating in maternal plasma presents the opportunity of a noninvasive method to prenatal diagnosis(72, 76).

Therefore, a new approach for the prenatal diagnosis of X-linked genetic disorders is currently possible and promising. With this approach, the sex of the fetus is defined by examination of maternal serum between 10 and 13 weeks of pregnancy, and if the fetus is identified

as male applied by chorionic villus sampling. If the fetus is recognized as female, chorionic-villus sampling is not applied, and fetal sex is approved later in the pregnancy by a new method known as ultrasonography.

Conclusion

Early diagnosis of prenatal diseases and cancer make available more potential for full rehabilitation and recovery of patients. Unfortunately, traditional methods of prenatal diagnosis screening are often invasive and expensive. Specificity and Sensitivity of these methods are also inadequate for prenatal diagnosis at an earlier stage. For this reason, many scientists are focusing to increase specificity and sensitivity of methods for early monitoring and detection of prenatal diseases. In the last decades, there has been a revolution in the number of studies analyzing cfDNA of maternal blood circulating, as a reliable and promising substitute for other invasivemethods and a promising tool for the prognosis and diagnosis of prenatal disease. At the present time, the main advantage of cfDNA as a reliable method is the easy availability and its stability in serum or plasma specimens.

Authors' contributions:

SG conceived of the study and participated in its design and coordination. GB, RN and SA participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

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Declaration of interest

The authors have no declaration of interest. The authors alone are responsible for the content and writing of the paper.

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