Review Article

The diagnostic potential of maternal plasma in detecting fetal diseases by DNA test

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Introduction

There is a common perception that fetal blood remains separated from maternal blood until third stage of labor or following abortion, inversion of placenta etc and only IgG can cross the placental barrier due to its small molecular weight. However, recent observations suggest that the placental barrier is not as impermeable as is traditionally known. This is because fetal DNA has been detected in maternal blood as early as 5 weeks.1 Real time PCR can be used to measure the concentration of fetal DNA in maternal plasma.2,3 Sex determining region of the Y chromosome (SRY) is a unique feature of a male. Normally, maternal DNA does not contain SRY. Hence, demonstration of SRY by PCR in maternal plasma during pregnancy indicates existence of a male fetus. Fetal sex determination is obviously useful for X linked recessive disorders. Detection of paternally inherited X chromosome polymorphism4 and molecular biology in the field of prenatal diagnosis.

As a standard practice, DNA based investigations are done from tissues obtained by chorionic villous sampling (CVS) or cells by amniocentesis. Both are invasive techniques requiring expertise to perform and carry little but definite risks. In pregnancy, investigation from a small amount of maternal blood is definitely a noninvasive technique. An early, noninvasive, reliable and cost-effective diagnostic investigation although generally desirable in medical practices, is particularly relevant to the prenatal diagnosis. Analyses of fetal DNA from maternal plasma / serum can provide an early prenatal diagnosis which conventional investigations fail to offer. A brief account of DNA based investigations from maternal blood has been given in this review.

Diagnostic usefulness

Maternal plasma is a chimeric mixture of fetal and maternal DNA. Real time PCR can be used to measure the concentration of fetal DNA in maternal plasma.2,3 Sex determining region of the Y chromosome (SRY) is a unique feature of a male. Normally, maternal DNA does not contain SRY. Hence, demonstration of SRY by PCR in maternal plasma during pregnancy indicates existence of a male fetus. Fetal sex determination is obviously useful for X linked recessive disorders. Detection of paternally inherited X chromosome polymorphism4 and molecular biology in the field of prenatal diagnosis.

1. Conventional DNA based investigations for fetal diseases are done by chorionic villous sampling and amniocentesis. Both are invasive techniques. Recently, molecular diagnosis has also been made possible in early pregnancy from maternal blood which is noninvasive and advantageous. Most of the researches have tried to identify the Y chromosome marker(s) to detect a male fetus and paternally inherited allele. This is currently helpful to detect a very few genetic disorders including Rh D status in Rh negative women in early pregnancy and preeclampsia a few weeks preceding the clinical onset. This is a potential area for prenatal diagnosis in future.

Key Words: Prenatal diagnosis, maternal plasma, DNA test, fetal diseases, PCR.
aneuploidy of female fetus by informative short tandem repeat (STR) markers have been made possible from maternal plasma. Apart from fetal Rh D status determination of the fetus in Rh D negative pregnant women, paternally inherited microsatellite polymorphism of chromosome 13, 18 and 21 have been detected. Prenatal diagnosis of autosomal recessive disorders (cystic fibrosis, congenital adrenal hyperplasia) and autosomal dominant disorders (myotonic dystrophy, achondroplasia, Huntington disease) have also been made.

Prenatal diagnosis in any pregnancy with the possibility of carrying an affected fetus is desired – the earlier, the better. Prenatal diagnosis can be offered in these cases noninvasively from maternal blood without any complication associated with CVS or amniocentesis.

Source of fetal DNA in maternal blood

Fetal DNA in maternal blood comes from continuous leakage of fetal cells (trophoblast, nucleated red blood cells, lymphocytes, granulocytes and stem cells) across the placenta with subsequent physical and immunological damage including death of fetal cells, active remodeling of the placenta with continuous cell lysis and developmentally associated apoptosis of fetal cells. Fetal DNA increases progressively as the pregnancy advances from about 3.4% at 11-17 weeks to about 6.2% at 37-43 weeks of the total maternal plasma DNA. The mean SRY concentration in maternal plasma collected at early pregnancy was 25.4 genome equivalents as opposed to a mean of 292.2 genome equivalents at late pregnancy. SRY level has been shown to peak at 33-36 weeks of pregnancy. The reasons could be increase in size of the fetus, placenta and fetomaternal interface as gestation progresses including a possible reduction in DNA clearance associated with other physical changes during pregnancy. There is a sharp increase in fetal DNA in the last 8 weeks of pregnancy which could be due to peak phase of fetal growth or breaking down of the placental barrier. Trophoblasts may be the predominant cell population involved in the liberation fetal DNA in the cell free fractions while fetal erythroblasts constitute the predominant fetal cell population in maternal blood.

Fate of fetal DNA

Fetal DNA is rapidly cleared from maternal plasma. Mean half-life of fetal DNA is 16.3 min (range 4 – 30 min). The clearance of DNA is mainly done by the kidney.

Fetal cells in maternal blood versus fetal DNA in maternal plasma

The average number of fetal cells in maternal blood is rare - 1.2 cells/ml of blood in 2nd trimester. The optimum week for prenatal diagnosis is around 15 weeks when maximum number of fetal nucleated red blood cells are found. About 8-20 ml blood is required. Fetal cell are cleared from maternal circulation within weeks with a half-life of 25-35 days. However, a cell subpopulation has been shown to persist for upto decades after delivery. Experiments with fetal cells require isolation and enrichment. The techniques are not only labor intensive and time-consuming (72 hrs) but also require costly equipments and thus, difficult to implement on a large scale.

In contrast, maternal plasma based DNA investigations are much simpler, robust and can be done around 7 weeks thereby offering the advantages of early diagnosis. Only 0.5 - 1 ml blood is required for the investigation. Fetal DNA from maternal plasma is cleared much more rapidly than fetal cells in maternal blood. Fetal DNA may be found in maternal plasma indefinitely after pregnancy in about one-fifth cases. However, other study showed that fetal DNA from previous pregnancy can not be detected in maternal serum even by using highly sensitive technique. This aspect has an important bearing while assessing future pregnancy(ies).

Recently, apoptotic fetal cells in maternal plasma have been utilized in the diagnosis of Down’s syndrome by fluorescent-in-situ-hybridization (FISH). This obviates the need for fetal cell enrichment procedure.

Maternal plasma versus maternal serum

The absolute concentration of fetal DNA in maternal plasma and serum is same but the relative amount of fetal DNA is less in serum than in plasma. This is due to more background maternal DNA resulting from cellular lysis occurring during coagulation. Hence, plasma is
preferred to serum for conventional PCR. Also, plasma can be readily obtained after blood collection by centrifugation.

Discussion

DNA based investigations result in molecular diagnosis of a disorder.

A number of studies have been conducted after the observation of fetal DNA in maternal plasma and serum for detecting fetal sex by Lo et al\textsuperscript{18} seven years ago. Only 0.01 ml of plasma was required for that purpose. Their group has been carrying out pioneering researches in this field.

Apart from SRY, there are several Y chromosome specific sequences e.g. DYZ1, DYZ3, DYS14, amelogenin like gene on Y (AMELY), zinc finger protein Y encoded (ZFY) etc. Among these, DYS14 had been frequently used in the past and DYZ3 having tandem repeat units was suspected to be highly sensitive in the detection of male fetal DNA.\textsuperscript{19} A study (n=263) was conducted in which the SRY sequence was never detected in women, detected in all pregnant women with male fetus and did not show any evidence of SRY when the previous child had been male but the current fetus was female.\textsuperscript{2} Interesting observations were also made from the serum using DYZ3 and DYS14. In one study (n=61) involving 10-17 weeks gestation, 100% fetal male sex was determined from serum while 87% from plasma.\textsuperscript{19} In another study (n=302), with a gestation of 7-16 weeks, a sensitivity of 97.2% and a specificity of 100% were obtained using maternal plasma. The false negative results yielded correct results after reanalysis\textsuperscript{20}. A conventional PCR identifies a male fetus with a total sensitivity of 95% (n=81, 5-10 weeks gestation) but its sensitivity after 7\textsuperscript{th} week is 100% whereas in real time quantitative PCR, the total sensitivity after 5\textsuperscript{th} week is 100%.\textsuperscript{21} Regarding determination of male sex, other studies showed a sensitivity ranging from about 89% - 97%\textsuperscript{22-24} to 100%.\textsuperscript{22,25-27} Occasionally, false positivity was noted.\textsuperscript{28,29} These discrepancies could be due to differences in the method of DNA preparation (extraction efficiency), low concentration of fetal DNA in early weeks of gestation, the primers used, differences in the DNA sequence targeted for amplification, technical perfection, absence of PCR inhibitors in the purified DNA, improved sensitivity of nested PCR, possibility of contamination etc.

Abnormal pregnancies associated with placental damage cause release of increased fetal cells and liberation of free DNA directly from dying cells in the placenta to the maternal blood e.g. aneuploid pregnancy (trisomy 21), toxemia of pregnancy, preterm labor, hyperemesis gravidarum etc.\textsuperscript{30-34} A few weeks before the onset of preeclampsia increased fetal erythroblastic trafficking, increased fetal DNA concentration, even increased maternal DNA itself have been observed.\textsuperscript{3,35} Therefore, increased circulating maternal DNA may precede the clinical manifestations of preeclampsia by a few weeks. Hence, Fetal DNA could be used as a dynamic marker of fetomaternal well being due to its lack of persistence and rapid clearance.

To perform DNA based investigations using PCR techniques is not as difficult as is commonly perceived. Also, commercial availability of kits has made the technical steps of DNA extraction easier than the laborious and time-consuming procedure.

The discovery of fetal DNA in maternal plasma and the demonstration of the relative ease and reliability with which it can be detected have opened up new possibilities for noninvasive prenatal diagnosis. Techniques have centered largely on sex determination and paternally inherited alleles for autosomal dominant disorders of the fetus. However, positive identification of paternally inherited normal allele would indirectly help in diagnosing autosomal recessive disease. The greatest practical problem was to differentiate fetal DNA in maternal plasma from maternal DNA. However, the quest to solve the above has been achieved to some extent by the recent detection of epigenetic markers in maternal plasma based on differential methylation of the selected gene locus.\textsuperscript{36} At the IGF-H19 locus, the maternally inherited fetal allele is unmethylated which can be differentiated by bisulfite conversion and methylation specific PCR from the paternally inherited allele which is methylated. Differentially methylated fetal alleles were subsequently detected by direct sequencing and primer extension assay. Undoubtedly, this is a potential area for future research and applications.

Keeping aside the possible misuse of only fetal sex
determination by unscrupulous persons in clandestine ways, with the research works done so far at least a part of the genetic disorders including preeclampsia can be diagnosed in early pregnancy for effective management well in advance. Fetal Rh D status has been predicted with 99.5% accuracy. Clinical service in connection with determination of Rh D status of the fetus in Rh D negative women has already been started. In case of congenital adrenal hyperplasia, early prenatal diagnosis can shorten the maternal dexamethasone therapy so as to prevent the virilization of the female fetus. Another very recent development is 100% detection of male pregnancy from dried maternal blood spots. This appears to be a robust method for collecting and transporting from remote places and hence would be very much useful for large scale study and wider applications. Recently, plasma mRNA marker has provided a gender independent approach. Study with hPL mRNA revealed that mRNA transcripts of placenta-expressed gene had unidirectional transfer from placenta into maternal plasma, were readily detectable, surprisingly stable and rapidly cleared after delivery. The availability of plasma mRNA based markers would open many new diagnostic opportunities and new understanding of fetomaternal physiology and pathology. Microarray technology will allow the very rapid development of new plasma RNA markers which can be used for noninvasive fetal gene expression profiling and prenatal monitoring.

The potential in the field of prenatal molecular diagnosis is, indeed, very vast with a greater impact in the management of high risk cases than previously. Active researches in this field are expected to fill up the deficiencies. Keeping in view of the spate of developments, it may be optimistically commented that in the near future, DNA based investigations will have a breakthrough in the day to day routine clinical prenatal diagnosis replacing the traditional invasive methods.

References


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