Use of cell-free fetal nucleic acids in maternal blood for prenatal diagnosis: the reality of this scenario in Brazil

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Summary

The discovery of cell-free fetal nucleic acids in the plasma of pregnant women has allowed the development of new, noninvasive prenatal diagnostic tests for the determination of fetal gender and Rh. These tests have been implemented in the public health system in several countries of Europe for over five years. The new possibilities for diagnostic use of these technologies are the detection of fetal chromosomal aneuploidies, monogenic fetal disorders, and placental-related disorders, subjects that have been intensively studied by several groups around the world. The aim of this review was to assess the Brazilian research and clinical scenarios regarding the utilization of commercially available tests that use these plasma markers, stressing the advantages, both economic and safety-related, that non-invasive tests have when compared to those currently used in the Brazilian public health system.

Keywords: Noninvasive diagnosis; prenatal care; free nucleic acids; fetal DNA; maternal plasma; Brazil.

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Introduction

The development and improvement of noninvasive diagnostic tests are consistently pursued goals in medicine. The discovery of cell-free fetal DNA in the blood of pregnant women[1], associated with the extreme sensitivity of molecular biology techniques that can detect very small amounts of nucleic acids, has enabled the development of new assays for noninvasive prenatal diagnosis (PND).

Research shows that cell-free fetal DNA comprises about 6% of the total amount in maternal circulation[2], and is completely eliminated after the delivery[3] (half-life of 16 minutes). These nucleic acids are released mainly by apoptosis of the trophoblast cells that form the placenta[4-6]. Their isolation from maternal blood is relatively simple, inexpensive, and adaptable to the routine of clinical laboratories.

The main technical difficulty of this type of analysis is the differentiation between cell-free fetal DNA and cell-free maternal DNA, which is present in plasma at higher amounts than the former; this differentiation is only possible when fetal genes or DNA segments with sequences that are different from the maternal genotype are analyzed.

In addition to DNA, messenger RNAs (mRNAs)[7] and microRNAs (short sequences of RNAs with post-transcriptional regulatory function)[8] of fetal origin also circulate in the maternal plasma. The analysis of these types of nucleic acids has an advantage over DNA analysis, as it allows the evaluation of fetal gene expression, which may reflect not only genetic characteristics (DNA) of the fetus, but momentary physiological situations[9,10].

The aim of this review was to assess the Brazilian scenario regarding noninvasive prenatal diagnostic testing, emphasizing tests that are already available in clinical practice in several countries, and to explore what is still possible to develop from the analysis of cell-free fetal nucleic acids (cffNA) in the plasma of pregnant women.

Diagnostic tests used in prenatal routine assessment

Fetal gender

Detection of Y chromosome sequences in maternal plasma indicates that the gender of the fetus is male, as normal women do not have the Y chromosome in their genome. In pregnant women in whom these sequences are not detected, the fetus is presumed female. Fetal gender determination was the first clinically available test that used analysis of fetal nucleic acids in maternal blood[11].

Most available tests detect the gender-determining region (SRY) of chromosome Y[12], although other Y-chromosome-specific sequences have been described, such as DYS[13-15], DYZ[16], and DAZ[17].

Over the years, the test has been improved, currently reaching over 99% of sensitivity and specificity after the 6th week of gestation, and reaching over 99.9% after the 8th week. There are some tools that ensure the safety of the test, such as the use of internal controls to prevent false-negative results (false evaluation of female fetal gender)[18].

Aside from the curiosity to know the fetal gender, the main clinical application of fetal gender determination is to aid in the investigation of genetic X-chromosome-linked diseases[19,20], such as Duchene's muscular dystrophy and hemophilia, where the confirmation of the female gender excludes the possibility of the fetus carrying such diseases, or even to indicate the treatment of metabolic disorders associated with ambiguous genitalia, such as congenital adrenal hyperplasia (CAH)[12].

In countries such as China, this testing is prohibited for ethical reasons[21], unless the knowledge of fetal gender offers the possibility of intrauterine treatment. Some European countries offer the test for free within the public health system for the investigation of gender-related diseases[22]. In Brazil, this test was first described by Levi et al. in 2003[23]. Currently several private laboratories in Brazil offer the test commercially to families who wish to know the fetal gender from the seventh week of pregnancy.

With technological development, the costs of fetal gender determination have decreased[24], making testing more accessible, and opening new perspectives of management for X-chromosome-linked genetic diseases, as previously described, including through the Unified Brazilian Health System (Sistema Único de Saúde – SUS).

Fetal Rh

Noninvasive genetic fetal RhD genotype testings in RhD-negative mothers through detection of free fetal DNA in maternal plasma was first demonstrated in 1998 by Lo et al.[25]. Since then, more robust methods have been developed, and currently the test is offered in the public health system of many countries in Europe (such as Belgium, United Kingdom, France, the Netherlands, Germany, Spain) with high accuracy (sensitivity of 100% and specificity of 98.6%)[26]. The test has been studied by some groups in Brazil[27,28], and one of them was able to demonstrate its applicability in highly mixed race populations such as that of Brazil, with 100% accuracy[29].

In RhD-negative pregnant women, there is a 16% chance of sensitization to RhD antigen when carrying a RhD-positive fetus, and the consequent development of hemolytic disease of the newborn[30].

There is a prophylaxis against the maternal immune response, which consists of the administration of anti-D immunoglobulin, which binds and neutralizes fetal RhD antigens. In Brazil, this treatment is performed in all RhD-negative pregnant women during pregnancy, regardless of fetal genotype. As 40% of RhD-negative pregnant women carry fetuses that are also RhD negative,

prophylaxis in these cases is unnecessary. Additionally, the product is obtained from human blood (plasma-derived), with a theoretical risk of spreading infection and with the risk of sensitivity reactions[31].

Currently, few private laboratories provide this test commercially in Brazil. The implementation of the test in the Brazilian public health system is extremely important to prevent the risks associated with anti-D immunoglobulin prophylaxis. It would also be economical, as it would eliminate the costs of unnecessary prophylaxis and the testing for monitoring maternal sensitization, as demonstrated in other countries[32].

New possibilities of use

Chromosomal aneuploidies

There are two major lines of research on tests for noninvasive PND assays for fetal chromosomal aneuploidy. One is focused on the analysis of cell-free fetal DNA through extremely sophisticated techniques[33] such as digital polymerase chain reaction (PCR)[34] (counting each DNA molecule) and mass sequencing (simultaneous sequencing of millions of DNA molecules)[35,36], allowing, in theory, for a precise quantification of the small variations in the total free DNA (maternal + fetal), consequent to the presence of extra genetic material or lack thereof in relation to normal, depending on the aneuploidy. The two major limitations to the practical implementation of this technique are its high cost and the complexity of the involved in this technology, including sophisticated bioinformatics software, with the diagnosis being achieved only in experimental situations, where it required a very large initial blood volume and proportions of free fetal DNA that are not practically feasible.

The other line of research focuses on the analysis of fetal gene expression, in which fetal-specific mRNAs (mainly expressed by placental tissue) are isolated from maternal plasma. The test is based on the single nucleotide polymorphism (SNP)/mRNA approach, where the ratio between the alleles of SNPs located in genes expressed by placental tissue allows the detection of extra copies of the chromosome where the investigated genes are located[37-39]. The frequency of heterozygous polymorphisms varies among different populations, so the test should use polymorphisms that are informative for each ethnic group.

Three major studies that investigated the effectiveness of tests that analyze fetal DNA using mass sequencing were recently published, demonstrating high sensitivity and specificity[40-42]. However, as these tests were based on extremely expensive and sophisticated technologies that do not allow simultaneous analysis of many samples (low efficiency), and with a long delay to release the results, there is no possibility of applying them in individual and less specialized clinical laboratories. These tests would have to be performed in major centers that have the technology, at high costs.

Some experts indicate the SNP/mRNA approach as the most promising for noninvasive PND of chromosomal aneuploidies, as the methodology is simple enough to be employed in small clinical laboratories, the costs are reasonable, and the diagnosis is achieved using samples of maternal plasma with high yield, 92% sensitivity, and 100% specificity[43]. Large-scale studies are needed to confirm this hypothesis.

A private North-American company has been offering the test commercially in this country since March, 2012, promising results in ten days, based on the recently published results of a prospective study using mass sequencing of fetal DNA in maternal plasma, conducted with 532 samples, which reached 100% specificity in the most prevalent trisomies[44]. In Brazil, no studies have been published on noninvasive diagnosis of fetal chromosomal aneuploidy to date.

Monogenic diseases

The investigation of monogenic diseases with high prevalence, such as thalassemia in populations of Mediterranean origin, and cystic fibrosis in populations of European origin, should be, in theory, part of routine prenatal research. In cases of rare monogenic diseases, such as Huntington's disease, investigation should be conducted when there is a family history of the disease.

The noninvasive PND of monogenic diseases with dominant paternal inheritance is possible due to the absence of the disease-causing allele in the maternal genome, and, so far, has been performed in at least one pregnancy for the following diseases: Huntington's disease[45-47], achondroplasia[48-50], myotonic dystrophy[51], and Apert syndrome[52].

Autosomal recessive diseases are often difficult to diagnose noninvasively, as there is still no way to distinguish between identical maternal and paternal alleles. Therefore, the cell-free fetal DNA in maternal plasma can only be used to determine the carrier status of the fetus in compound heterozygotes, by detecting the disease allele inherited from the father (in cases where more than one allele is known for such disease), and when the maternal and paternal alleles are different. To date, the carrier status of the fetus has been determined using cell-free fetal DNA in the following recessive genetic diseases, in at least one pregnancy: cystic fibrosis[53-55], β-thalassemia[56], α-thalassemia 1[57], congenital adrenal hyperplasia[58], and hemophilia[59] in some European countries. There have been no studies or case reports in Brazil using cffNA for the noninvasive diagnosis of autosomal recessive diseases, or monogenic diseases of paternal inheritance.

Placental-related disorders

Recent literature has proposed that cffNA can be used to predict disorders whose etiology is based on poor placental development, as this is associated with increased apoptosis of the trophoblast cells that form the outermost placental layer[5].

Several groups have associated an increase in the amount of cffNA in maternal plasma with disorders whose etiology seems to be related to poor placental development. Some examples are preeclampsia[60-62], HELLP syndrome[63], fetal growth restriction[64,65], premature birth[66,67], placenta accreta[68], placenta inccreta[69], hyperemesis gravidarum[70], and polyhydramnios[71]. In some cases, it has been verified that there is an increase in cffNA levels before symptom onset, and that these levels correlate with disease severity.

The major limitation of using cffNA as a marker of these placental disorders is that many of them have a multifactorial etiology, or are often unknown. It is first necessary to elucidate the nature of these diseases, as well as establish a normality curve of cffNA concentration in normal pregnancies, so that, later, the analysis in affected pregnancies can be carried out.

Conclusion

Noninvasive determination of fetal gender and Rh is well established and has been used clinically for more than five years by several countries in Europe[72-74]. In Brazil, these tests have limited use due to their high costs. Their implementation in the public health system would be extremely important as an alternative to invasive procedures, as the latter bring risks during pregnancy and ultimately are outdated when compared to the safety and accuracy of the new tests. Furthermore, the use of noninvasive tests can be advantageous from an economic viewpoint, as the value of a noninvasive test tends to be lower than that of invasive tests, and the early result prevents unnecessary Rh prophylaxis costs in Rh-negative pregnant women and therapy for CAH in male fetuses.

Brazil has a great potential for the development of pioneering and innovative PND research, as recent data from the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística – IBGE) show that there are 2.5 million births per year in the country and an even larger number of pregnancies, both healthy and affected by different complications. This potential should be explored by the research groups specializing in each type of pregnancy complications (such as preeclampsia, fetuses with aneuploidy, monogenic diseases, among others), so that the results of these studies are given back to society as safer and more effective tests and made available in the public health system.

References

1. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS: Presence of fetal DNA in maternal plasma and serum. Lancet. 1997;350:485-7.
2. Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet. 1998;62:768-75.
3. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet. 1999;64:218-24.
4. Bischoff FZ, Lewis DE, Simpson JL. Cell-free fetal DNA in maternal blood: kinetics, source and structure. Hum Reprod Update. 2005;11:59-67.
5. Flori E, Doray B, Gautier E, Kohler M, Ernault P, Flori J, et al. Circulating cell-free fetal DNA in maternal serum appears to originate from cyto- and syncytio-trophoblastic cells. Case report. Hum Reprod. 2004;19:723-4.
6. Lichtenstein AV, Melkonyan HS, Tomei LD, Umansky SR. Circulating nucleic acids and apoptosis. Ann N Y Acad Sci. 2001;945:239-49.
7. Poon LL, Leung TN, Lau TK, Lo YM. Presence of fetal RNA in maternal plasma. Clin Chem. 2000;46:1832-4.
8. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem. 2008;54:482-90.
9. Farina A, Chan CW, Chiu RW, Tsui NB, Carinci P, Concu M, et al. Circulating corticotropin-releasing hormone mRNA in maternal plasma: relationship with gestational age and severity of preeclampsia. Clin Chem. 2004;50:1851-4.
10. Ng EK, Leung TN, Tsui NB, Lau TK, Panesar NS, Chiu RW, et al. The concentration of circulating corticotropin-releasing hormone mRNA in maternal plasma is increased in preeclampsia. Clin Chem. 2003;49:727-31.
11. Sekizawa A, Kondo T, Iwasaki M, Watanabe A, Jimbo M, Saito H, et al. Accuracy of fetal gender determination by analysis of DNA in maternal plasma. Clin Chem. 2001;47:1856-8.
12. Rijnders RJ, van der Schoot CE, Bossers B, de Vroede MA, Christiaens GC. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. Obstet Gynecol. 2001;98:374-8.
13. Honda H, Miharu N, Ohashi Y, Samura O, Kinutani M, Hara T, et al. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. Hum Genet. 2002;110:75-9.
14. Zimmermann B, El-Sheikhah A, Nicolaides K, Holzgreve W, Hahn S. Optimized real-time quantitative PCR measurement of male fetal DNA in maternal plasma. Clin Chem. 2005;51:1598-604.
15. Deng Z, Wu G, Li Q, Zhang X, Liang Y, Li D, et al. Noninvasive genotyping of 9 Y-chromosome specific STR loci using circulatory fetal DNA in maternal plasma by multiplex PCR. Prenat Diagn. 2006;26:362-8.
16. Honda H, Miharu N, Ohashi Y, Ohama K. Successful diagnosis of fetal gender using conventional PCR analysis of maternal serum. Clin Chem. 2001;47:41-6.
17. Stanghellini I, Bertorelli R, Capone L, Mazza V, Neri C, Percesepe A, et al. Quantitation of fetal DNA in maternal serum during the first trimester of pregnancy by the use of a DAZ repetitive probe. Mol Hum Reprod. 2006;12:587-91.
18. Chiu RW, Lo YM. Non-invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the coming of age. Semin Fetal Neonatal Med. 2011;16:88-93.
19. Hyett JA, Gardener G, Stojilkovic-Mikic T, Finning KM, Martin PG, Rodeck CH, et al. Reduction in diagnostic and therapeutic interventions by non-invasive determination of fetal sex in early pregnancy. Prenat Diagn. 2005;25:1111-6.
20. Sekizawa A, Saito H. Prenatal screening of single-gene disorders from maternal blood. Am J Pharmacogenomics. 2001;1:111-7.
21. Newson AJ. Ethical aspects arising from non-invasive fetal diagnosis. Semin Fetal Neonatal Med. 2008;13:103-8.
22. Hill M, Finning K, Martin P, Hogg J, Meaney C, Norbury G, et al. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. Clin Genet. 2011;80:68-75.
23. Levi JE, Wendel S, Takaoka DT. Determinação pré-natal do sexo fetal por meio da análise de dna no plasma materno. Rev Bras Ginecol Obstet. 2003;25:687-90.
24. Hill M, Taffinder S, Chitty LS, Morris S. Incremental cost of non-invasive prenatal diagnosis versus invasive prenatal diagnosis of fetal sex in England. Prenat Diagn. 2011;31:267-73.
25. Lo YM, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med. 1998;339:734-8.
26. Macher HC, Noguerol P, Medrano-Campillo P, Garrido-Marquez MR, Rubio-Calvo A, Carmona-Gonzalez M, et al. Standardization non-invasive fetal RHD and SRY determination into clinical routine using a new multiplex RT-PCR assay for fetal cell-free DNA in pregnant women plasma: results in clinical benefits and cost saving. Clin Chim Acta. 2012;413:490-4.
27. Machado IN, Castilho L, Pellegrino Jr J, Barini R. Fetal rhd genotyping from maternal plasma in a population with a highly diverse ethnic background. Rev Assoc Med Bras. 2006;52:232-5.
28. Chinen PA, Nardozza LM, Martinhago CD, Camano L, Daher S, Pares DB, et al. Noninvasive determination of fetal rh blood group, D antigen status by cell-free DNA analysis in maternal plasma: experience in a Brazilian population. Am J Perinatol. 2010;27:759-62.

29. Amaral DR, Credidio DC, Pellegrino Jr J, Castilho L. Fetal RHD genotyping by analysis of maternal plasma in a mixed population. J Clin Lab Anal. 2011;25:100-4.
30. Bowman J. Thirty-five years of Rh prophylaxis. Transfusion. 2003;43:1661-6.
31. Van der Schoot CE, Hahn S, Chitty LS. Non-invasive prenatal diagnosis and determination of fetal Rh status. Semin Fetal Neonatal Med. 2008;13:63-8.
32. Benachi A, Delahaye S, Leticee N, Jouannic JM, Ville Y, Costa JM. Impact of non-invasive fetal RhD genotyping on management costs of rhesus-D negative patients: results of a French pilot study. Eur J Obstet Gynecol Reprod Biol. 2012;162:28-32.
33. Chiu RW, Cantor CR, Lo YM. Non-invasive prenatal diagnosis by single molecule counting technologies. Trends Genet. 2009;25:324-31.
34. Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, Lau TK, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proc Natl Acad Sci USA. 2007;104:13116-21.
35. Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci USA. 2008;105:20458-63.
36. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci USA. 2008;105:16266-71.
37. Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med. 2007;13:218-23.
38. Tsui NB, Wong BC, Leung TY, Lau TK, Chiu RW, Lo YM. Non-invasive prenatal detection of fetal trisomy 18 by RNA-SNP allelic ratio analysis using maternal plasma SERPINB2 mRNA: a feasibility study. Prenat Diagn. 2009;29:1031-7.
39. Tsui NB, Akolekar R, Chiu RW, Chow KC, Leung TY, Lau TK, et al. Synergy of total PLAC4 RNA concentration and measurement of the RNA single-nucleotide polymorphism allelic ratio for the noninvasive prenatal detection of trisomy 21. Clin Chem. 2010;56:73-81.
40. Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ. 2011;342:c7401.
41. Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol. 2011;204:205-11.
42. Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem. 2011;57:1042-9.
43. Deng YH, Yin AH, He Q, Chen JC, He YS, Wang HQ, et al. Non-invasive prenatal diagnosis of trisomy 21 by reverse transcriptase multiplex ligation-dependent probe amplification. Clin Chem Lab Med. 2011;49:641-6.
44. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012;119:890-901.
45. Gonzalez-Gonzalez MC, Trujillo MJ, Rodriguez de Alba M, Garcia-Hoyos M, Lorda-Sanchez I, Diaz-Recasens J, et al. Huntington disease-unaffected fetus diagnosed from maternal plasma using QF-PCR. Prenat Diagn. 2003;23:232-4.
46. Bustamante-Aragones A, Trujillo-Tiebas MJ, Gallego-Merlo J, Rodriguez de Alba M, Gonzalez-Gonzalez C, Cantalapiedra D, et al. Prenatal diagnosis of Huntington disease in maternal plasma: direct and indirect study. Eur J Neurol. 2008;15:1338-44.
47. Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo-Tiebas MJ, Bustamante Aragones A, Rodriguez de Alba M, Diego Alvarez D, et al. Improvement in strategies for the non-invasive prenatal diagnosis of Huntington disease. J Assist Reprod Genet. 2008;25:477-81.
48. Li Y, Holzgreve W, Page-Christiaens GC, Gille JJ, Hahn S. Improved prenatal detection of a fetal point mutation for achondroplasia by the use of size-fractionated circulatory DNA in maternal plasma--case report. Prenat Diagn. 2004;24:896-8.
49. Li Y, Page-Christiaens GC, Gille JJ, Holzgreve W, Hahn S. Non-invasive prenatal detection of achondroplasia in size-fractionated cell-free DNA by MALDI-TOF MS assay. Prenat Diagn. 2007;27:11-7.
50. Chitty LS, Griffin DR, Meaney C, Barrett A, Khalil A, Pajkrt E, et al. New aids for the non-invasive prenatal diagnosis of achondroplasia: dysmorphic features, charts of fetal size and molecular confirmation using cell-free fetal DNA in maternal plasma. Ultrasound Obstet Gynecol. 2011;37:283-9.
51. Amicucci P, Gennarelli M, Novelli G, Dallapiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. Clin Chem. 2000;46:301-2.
52. Au PK, Kwok YK, Leung KY, Tang LY, Tang MH, Lau ET. Detection of the S252W mutation in fibroblast growth factor receptor 2 (FGFR2) in fetal DNA from maternal plasma in a pregnancy affected by Apert syndrome. Prenat Diagn. 2011;31:218-20.
53. Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo MJ, Rodriguez de Alba M, Lorda-Sanchez I, Diaz-Recasens J, et al. Prenatal detection of a cystic fibrosis mutation in fetal DNA from maternal plasma. Prenat Diagn. 2002;22:946-8.
54. Bustamante-Aragones A, Gallego-Merlo J, Trujillo-Tiebas MJ, de Alba MR, Gonzalez-Gonzalez C, Glover G, et al. New strategy for the prenatal detection/exclusion of paternal cystic fibrosis mutations in maternal plasma. J Cyst Fibros. 2008;7:505-10.
55. Nasis O, Thompson S, Hong T, Sherwood M, Radcliffe S, Jackson L, et al. Improvement in sensitivity of allele-specific PCR facilitates reliable noninvasive prenatal detection of cystic fibrosis. Clin Chem. 2004;50:694-701.
56. Chiu RW, Lau TK, Leung TN, Chow KC, Chui DH, Lo YM. Prenatal exclusion of beta thalassaemia major by examination of maternal plasma. Lancet. 2002;360:998-1000.
57. Sirichotiyakul S, Charoenkwan P, Sanguansermsri T. Prenatal diagnosis of homozygous alpha-thalassemia-1 by cell-free fetal DNA in maternal plasma. Prenat Diagn. 2012;32:45-9.
58. Chiu RW, Lau TK, Cheung PT, Gong ZQ, Leung TN, Lo YM. Noninvasive prenatal exclusion of congenital adrenal hyperplasia by maternal plasma analysis: a feasibility study. Clin Chem. 2002;48:778-80.
59. Tsui NB, Kadir RA, Chan KC, Chi C, Mellars G, Tuddenham EG, et al. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood. 2011;117:3684-91.
60. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. Clin Chem. 1999;45:184-8.
61. Zhong XY, Holzgreve W, Hahn S. The levels of circulatory cell free fetal DNA in maternal plasma are elevated prior to the onset of preeclampsia. Hypertens Pregnancy. 2002;21:77-83.
62. Levine RJ, Qian C, Leshane ES, Yu KF, England LJ, Schisterman EF, et al. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. Am J Obstet Gynecol. 2004;190:707-13.
63. Swinkels DW, de Kok JB, Hendriks JC, Wiegerinck E, Zusterzeel PL, Steegers EA. Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome as a complication of preeclampsia in pregnant women increases the amount of cell-free fetal and maternal DNA in maternal plasma and serum. Clin Chem. 2002;48:650-3.
64. Sekizawa A, Jimbo M, Saito H, Iwasaki M, Matsuoka R, Okai T, et al. Cell-free fetal DNA in the plasma of pregnant women with severe fetal growth restriction. Am J Obstet Gynecol. 2003;188:480-4.
65. Smid M, Galbiati S, Lojacono A, Valsecchi L, Platto C, Cavoretto P, et al. Correlation of fetal DNA levels in maternal plasma with Doppler status in pathological pregnancies. Prenat Diagn. 2006;26:785-90.
66. Leung TN, Zhang J, Lau TK, Hjelm NM, Lo YM. Maternal plasma fetal DNA as a marker for preterm labour. Lancet. 1998;352:1904-5.
67. Farina A, LeShane ES, Romero R, Gomez R, Chaiworapongsa T, Rizzo, N et al. High levels of fetal cell-free DNA in maternal serum: a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol. 2005;193:421-5.
68. Sekizawa A, Jimbo M, Saito H, Iwasaki M, Sugito Y, Yukimoto Y, et al. Increased cell-free fetal DNA in plasma of two women with invasive placenta. Clin Chem. 2002;48:353-4.
69. Jimbo M, Sekizawa A, Sugito Y, Matsuoka R, Ichizuka K, Saito H, et al. Placenta increta: postpartum monitoring of plasma cell-free fetal DNA. Clin Chem. 2003;49:1540-1.
70. Sugito Y, Sekizawa A, Farina A, Yukimoto Y, Saito H, Iwasaki M, et al. Relationship between severity of hyperemesis gravidarum and fetal DNA concentration in maternal plasma. Clin Chem. 2003;49:1667-9.
71. Zhong XY, Holzgreve W, Li JC, Aydinli K, Hahn S. High levels of fetal erythroblasts and fetal extracellular DNA in the peripheral blood of a pregnant woman with idiopathic polyhydramnios: case report. Prenat Diagn. 2000;20:838-41.
72. Hill M, Finning K, Martin P, Hogg J, Meaney C, Norbury G, et al. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. Clin Genet. 2011;80:68-75.
73. Scheffer P, van der Schoot C, Page-Christiaens G, de Haas M. Noninvasive fetal blood group genotyping of rhesus D, c, E and of K in alloimmunised pregnant women: evaluation of a 7-year clinical experience. BJOG. 2011;118:1340-8.
74. Macher HC, Noguerol P, Medrano-Campillo P, Garrido-Marquez MR, Rubio-Calvo A, Carmona-Gonzalez M, et al. Standardization non-invasive fetal RHD and SRY determination into clinical routine using a new multiplex RT-PCR assay for fetal cell-free DNA in pregnant women plasma: results in clinical benefits and cost saving. Clin Chim Acta. 2012;413:490-4.