

Non-Invasive Prenatal Diagnosis in Twin Pregnancies: Current Status

Abstract

The objective of this review is to assess the evidence which supports the use of non-invasive prenatal diagnosis (NIPD) in twin pregnancies. Through the years, we have witnessed the technological developments in non-invasive prenatal diagnosis attained new heights, but those studies were usually limited in singleton pregnancies. As we known, twin pregnancies are at higher risk in both aneuploidy and structural abnormalities. The first- and second- trimester aneuploidy screening in twin gestations are less accurate than in singleton gestations. And the invasive prenatal diagnosis in twin pregnancies is associated with a high risk of pregnancy loss. So, it is urgent to develop accurate non-invasive prenatal diagnosis in twin pregnancies. Recent studies about different terminology and methods in NIPD of twin pregnancies have been reported, suggesting that non-invasive prenatal testing can be feasibly and reliably used in twin pregnancies. In this article, we summarized the published literature on non-invasive prenatal diagnosis in twin pregnancies in order to benefit our clinical practice.

Keywords: Non-invasive prenatal diagnosis (NIPD); Twin pregnancies; Maternal plasma DNA analysis; Zygosity; Aneuploidies

Review Article

Volume 4 Issue 5 - 2016

Kana Wang¹, Jian Zhang² and Haiyan Yu^{1*}

¹Department of Gynecology and Obstetrics, Sichuan University, China

²Department of Gynecology and Obstetrics, Sichuan Academy of Medical Sciences & Sichuan Province People's Hospital, China

***Corresponding author:** Haiyan Yu, Division of Perinatology and Fetal Medicine, University of Sichuan, West China Second University Hospital and West China Medical School, No. 20, 3rd section, South Renmin Road, Chengdu, Sichuan, 610041, China, Tel: +862885503735; Email: fanjy422@163.com

Received: May 16, 2016 | **Published:** June 21, 2016

Introduction

For more than 20 years, the published studies have described the successful use of non-invasive methods to detect fetal aneuploidy, fetal gender, single gene genetic disease, etc, in singleton pregnancies [1,2]. Those methods have made great progress from fetal nucleated cells to fetal genetic material in maternal plasma [3]. The existence of fetal derived cell-free DNA molecules in plasma of pregnant women was first demonstrated in 1997, this finding provided a new way for noninvasive prenatal diagnosis [4]. Because fetal cells could retain in maternal blood for almost 27 years, while the fetal cell-free DNA could be rapidly removed within a short period of time (2 hours postpartum), so the prenatal diagnosis could not be affected by past pregnancy. And the content of fetal cell-free DNA in maternal peripheral blood was higher than fetal DNA in nucleated cells; its specific sequences could be amplified and quantitatively analyzed by PCR, which made the separation and enrichment of fetal source DNA relatively simple [5-8].

Most recently, the discovery of fetal cell-free RNA in maternal plasma opened a new era for non-invasive prenatal diagnosis. Instead of fetal DNA, the cell-free RNA was shown to be feasible in more research of NIPD for Down syndrome, Edwards syndrome (trisomy 18) and other obstetric complications [9-13]. The published studies suggested that the cell-free RNA coming from placenta has a good stability in maternal blood and can be rapid removed after delivery [2,8]. And the fetal cell-free RNA reflected the gene expression, independence of gender and polymorphism [14].

Nowadays, those specific sequence detection in maternal plasma had been used for the detection of paternally inherited traits and the chromosome aneuploidy, identification of fetal gender or rhesus D status and single gene genetic disease [1,15]. And the changes in free DNA or RNA levels could also be used for pregnancy related diseases such as preeclampsia, premature delivery and fetal growth restriction [1]. Non-invasive prenatal diagnosis reduced the fetal loss, intrauterine infection rate, and the use of invasive test. However, there was still some limitation about failure rates and risk factors for failed NIPT on singleton pregnancies. There is also still limited evidence about the performance of NIPT as a test in twin or triplet pregnancies [16]. With the increased incidence of twin pregnancies, accurate and fast non-invasive prenatal diagnoses in twin gestations are urgently needed.

Ultrasonography in Prenatal Diagnosis of Twin Gestations

Ultrasound examination played a very important role in twin pregnancies, which could determine the chorionicity, verify the gestational weeks and screen for fetal anomalies by measure fetal nuchal translucency (NT) in the early trimester [17-19]. Chorionicity of twin pregnancy had a major impact on the outcome of twin pregnancies, and it was closely related with the prenatal diagnosis of chromosome aneuploidy abnormality [19,20]. According to the sign of placenta from the joint observed by ultrasonography in 11-13+6 weeks of twin pregnancies, dichorionic twin can be diagnosed with the "Lambda" sign; while the monochorionic twin with the "T" sign. At the same gestational

week, NT was also a significant index for screening the fetal chromosomal aneuploidy abnormalities. Because NT could be determined separately for each fetus of twin and its distribution did not show significant difference between twins and singletons, NT measurement combined with maternal age has been used for prenatal aneuploidy screening in twins with the sensitivity of 75% [21]. However, the prevalence of increased NT was higher in women with monochorionic pregnancies than in those with dichorionic pregnancies, suggesting that increased NT in monochorionic twins may be an early manifestation of the twin-twin transfusion syndrome [22]. Therefore, the aneuploidy risk calculated by NT should be adjusted in monochorionic twins [19].

In the second trimester, ultrasound scanning usually can find structural fetal anomalies, such as cardiac malformation, and neural tube malformation (NTD). It has been noticed that the soft marker could help to detect fetal chromosomal aneuploidy by the ultrasound screening. For example, the soft markers, like the thickening of the nuchal skinfold, the absent of nasal bone, brachycephaly, flat forehead, short eared, short humerus, and the soft marker can be found in Down syndrome fetus [23]. Unfortunately, there were few data about the accuracy in twins.

In addition, obstetric ultrasound are very important for both singleton and multiple gestations throughout the whole pregnancy, including placental evaluation, cervical length assessment, routine fetal growth, and serial surveillance of pregnancies complications, cervical shortening, fetal growth disturbances, and amniotic fluid abnormalities [24-26].

Maternal Serum Screening for Aneuploidy in Twins

In twin pregnancies, the second trimester maternal serum screening for aneuploidy was more completed difficult. Theoretically, the serum marker levels in twins should be twice those found in singleton pregnancies, and twin pregnancies should have double risk for aneuploidy. However, more and more recent studies found that the risk of aneuploidy in monochorionic twins appeared similar to that in singleton pregnancies [18,23,27]. And they also found that there were wide variations of the serum marker in twins, the distribution of serum markers in twin pregnancies was unknown. In addition, the serum biochemistry that related to the entire pregnancy could not be identified in which individual fetus to the analyses values [28]. Thus, the Clinical Practice Guideline in 2007 indexed that the first trimester NT combined with maternal age might be the optimal way to assess Down syndrome risk in patients with a multiple pregnancy. Only if NT screening was not available or had been missed because of the late diagnosis of a twin pregnancy (after 14 weeks), the second trimester maternal serum screening might be considered in twins [21]. Nevertheless, a recent report has been published about the benefit of first-trimester combined risk assessment of free beta-human chorionic gonadotrophin (β -hCG), pregnancy-associated plasma protein A (PAPP-A) and nuchal translucency for Down syndrome in twin pregnancies [29]. To date, it has been suggested that the first-trimester combined test in twins for Down syndrome had a high detection rate and an acceptable false-positive rate which was 5.7% and 4.4% in dichorionic and monochorionic twins, respectively.

For decades, the fetal ultrasound assessment and the measurement of maternal serum markers have been implemented

effectively in Down syndrome prenatal screening programs. However, because the gestational age must be taken into account, the time limitation limited the use of ultrasound diagnosis and serum screening in multiple pregnancies. With this combined measurement 3%~5% of screened women were still identified as high-risk and needed to undergo invasive diagnosis such as Amniocentesis (AC) or chorionic villus sampling (CVS), which may lead to intrauterine infection or fetal loss [30].

Noninvasive Prenatal test for Fetal Gender in Twin Pregnancies

It is well known that ultrasound scanning can help determine fetal gender at the second trimester. But ultrasound assessment of fetal sex has limited accuracy in the first trimester or usually affected by the fetal position [31]. Since fetal cell-free DNA in maternal plasma has been discovered, it has been widely investigated in the field of non-invasive prenatal diagnosis. The fetal cell-free DNA can also be used as a noninvasive method to determine the fetal sex without the limitation of gestational age [32-34]. Mortarino et al. [34] demonstrated that fetal gender determination in maternal plasma is reliable after the 9th week of singleton gestation. However, the noninvasive prenatal test in twin or multiple pregnancies was more complex and difficult. Study of SRY-specific cell free fetal DNA (SRY-cffDNA) suggested that the levels of SRY-cffDNA in maternal plasma of male twin pregnancies were significantly increased compared to singleton male pregnancies after 28 weeks [35]. Meanwhile, considering the multicopy sequence such as DYS14 might be achieved a greater sensitivity than the single-copy on the Y-chromosome, Attilakos et al. [36] tested the plasma concentration of DYS14 in singleton and twin pregnancies at 18-20 week pregnancies. Their results shown that cfDNA concentration in two male pregnancies were significantly higher than that with one male fetus. Recently, Picchiasse et al. [37] detected the DYS14-cfDNA concentration in multiple pregnancies between 11-14 weeks of gestation, and they found it correctly predicted fetal gender, distinguishing twin pregnancies with at least one male fetus with a diagnostic accuracy of 100%.

Noninvasive Prenatal Determination of Twin Zygosity by Maternal Plasma DNA Analysis

Determine the zygosity of the twin pregnancies can be considered as a quality control step to the overall noninvasive prenatal diagnosis of twins [38,39]. Ultrasound examination could accurately determine chorionicity but not zygosity, the maternal plasma DNA analysis could be used for screening the zygosity instead [39,40]. In dichorionic twins the majority of cases are dizygotic. And the monozygotic twins, can clinically present as monochorionic twin, generally have a higher risk of obstetric complications than dizygotic twins. But the dizygotic twins need to be assessed individually, because the dizygotic pregnancy was derived from two fertilized eggs and the fetal DNA fraction was contributed by each individual twin member; while the monozygotic twins could be assessed like singleton pregnancies. When the monozygotic and dizygotic twins have been distinguished, fetal fraction (FF) need to be further estimated by polymorphic alleles using Y-chromosome sequences [40]. A lower FF could lead to a false negative result of cfDNA and the minimum fetal fraction required for aneuploidy assessment with

current methods is 4% [41,42]. Accordingly, Struble's et al. [40] study showed that the median total FF was 14.0% in monozygotic twins and the median was 7.9% in dizygotic twins [40].

Non-invasive Prenatal Diagnosis for Fetal Chromosomal Aneuploidies by Sequencing of Maternal Plasma DNA in Twin Pregnancies

Non-invasive prenatal testing (NITP) for fetal chromosomal abnormalities in singleton pregnancies has enormously improved by measuring fetal cell-free DNA in maternal plasma, which can detect nearly all cases of Down syndrome with a very low false-positive rate. According to the study of Palomaki et al. [43] Down syndrome detection rate was 98.6%, and the false-positive rate was 0.2% [43]. Subsequent research further reported that NIPT is extremely reliable with sensitivities and specificities greater than 99% [44,45]. Moreover, with the non-invasive method, trisomy 18 and other chromosomal abnormalities also could be identified

but the sensitivity was lower than Down syndrome [42,44,46-47]. Most recently, few studies have focused on the non-invasive prenatal diagnosis of twin or multiple pregnancies. The published data (Table 1) shown that NITP of fetal chromosomal aneuploidy for twin or multiple pregnancies could be achieved with the use of sequencing of maternal plasma DNA [16,38,41,48-49]. Those studies mostly used samples at 12 weeks' gestation or beyond. False-positive rate on cases before 12 weeks of gestation remained unexplained. In general, the measurement was low false-positive rate and related high detection rate as same as in singleton pregnancies [42,44]. Because there was an overlap between the fetal free DNA concentration levels of multiple pregnancies, so that the affected fetus could not be easily identified. It was therefore possible in the future to determine which of the two fetuses was affected by NIPT [16]. Although the data from prospective studies [34-39,44] has demonstrated that noninvasive determination of zygosity and fetal sex may possibly offer assistance, the findings of twin pregnancies need more confirmation in further researches.

Table 1: Published studies regarding measurement fetal DNA of maternal plasma for fetal chromosomal aneuploidies in twin or multiple pregnancies.

Author (year)	Cases no.	Test Methods	Gestational Age	Trisomy 21(n)	Trisomy 18(n)	Trisomy 13(n)	False-positive Rate	Sensitivity and Specificity
Canick JA et al. [48] (2012)	25	DNA sequencing of maternal plasma	Secondary Pregnancy (after 12 week of gestation)	7	0	1	None	100%
Leung TY et al. [38] (2012)	8	MPS of cell-free DNA	11 to 36 weeks	1	1	Nd	Nd	Nd
Lau TK et al. [49] (2013)	12	Maternal plasma DNA sequencing	Secondary Pregnancy	1	Nd	Nd	Nd	Both 100 %
del Mar Gil M et al. [41] (2013)	68	Sequencing of cell-free DNA in maternal blood	10 to 13 weeks	10	1	3	None	> 99%
Huang X et al. [16] (2014)	189	MPS of maternal plasma DNA	13 to 28 weeks	9	1	Nd	Nd	T21: both 100% T18: 50% and 100%, respectively

MPS: Massively Parallel Sequencing; None: no false positive results; Nd: Not Described; T21: Trisomy 21; T18: Trisomy 18

Noninvasive Approaches to Prenatal Diagnosis of Pregnancy Complications

Maternal serum analyzing is a noninvasive test of placental biochemical function. In the second and the third trimester, the pregnancy complications with placental dysfunction such as preeclampsia, fetal growth restriction and hemoglobinopathies, can also be estimated by analyzing the fetal free DNA levels of maternal plasma [49,50]. The fetal DNA not only increased with gestational age, but also appeared different between the severe placental dysfunction and milder [51]. The dysfunctional degree most characterized by impaired trophoblastic invasion of the maternal spiral arteries. The detection of free fetal DNA could be a potential prognostic marker for placental dysfunction [52]. In addition, the level of free fetal DNA also correlated with the degree

of placental injury which could predict the pregnancy outcome. In the research of Wataganara et al. [53] fetal DNA in maternal plasma was quantified by polymerase chain reaction amplification of Y-chromosome sequence [53]. And the result suggested that circulating fetal DNA could be derived from placental injury after laser thermocoagulation for twin-twin transfusion syndrome (TTTS), which can caused increased cell-free fetal DNA levels in maternal plasma.

Circulating microRNAs in Maternal Plasma for Non-invasive Prenatal Diagnosis of Twins

As mentioned before, the fetal cell-free RNA in maternal plasma may open a new way for non-invasive prenatal diagnosis. It has more advantages than fetal cell-free DNA [2,14]. But till

now, only a little study has been reported about the detection of fetal cell-free RNA for twin or multiple pregnancies. In the research of Ge et al. [54], they found that several circulating micro RNAs in maternal plasma were validated that remarkably changed in twin pregnancy, and suggested that miRNAs might involve the process of pregnancy such as the generation of twin pregnancy, for instance, mir-451 might regulate the embryo cell differentiation during embryogenesis of twin pregnancy. Their data also suggested the specific miRNAs could act as potential biomarkers for clinical diagnosis and the therapy of pregnancy complications such as preeclampsia.

Conclusion

Ultimately, this review showed that NIPD for twin pregnancies was necessary and feasible. Non-invasive prenatal testing could be used to determinate zygosity, distinguish fetal gender, to detect chromosomal abnormalities and other obstetric complications in twin pregnancies. However, till now, there were only a few convictive studies on fetal chromosomal aneuploidy of twin or multiple gestations by testing maternal plasma fetal cell-free nucleotide as that in singleton pregnancies. Further more studies are required.

References

- Chiu RW, Lo YM (2013) Clinical applications of maternal plasma fetal DNA analysis: translating the fruits of 15 years of research. *Clin Chem Lab Med* 51(1): 197-204.
- Tsui NB, Ng EK, Lo YM (2002) Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin Chem* vol 48(10): 1647-1653.
- Hahn S, Zhong XY, Holzgreve W (2008) Recent progress in noninvasive prenatal diagnosis. *Semin Fetal Neonatal Med* 13(2): 57-62.
- Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, et al. (1997) Presence of fetal DNA in maternal plasma and serum. *Lancet* 350(9076): 485-487.
- Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, et al. (1999) Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 64(1): 218-224.
- Dhallan R, Guo X, Emche S, Damewood M, Bayliss P, et al. (2007) A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 369(9560): 474-481.
- Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, et al. (2007) Digital PCR for the molecular detection of fetal chromosomal neuploidy, *Proc Natl Acad Sci USA* 104(32): 13116-13121.
- Poon LL, Leung TN, Lau TK, Chow KC, Lo YM (2002) Differential DNA methylation between fetus and mother as a strategy for detecting fetal DNA in maternal plasma. *Clin Chem* 48(1): 35-41.
- Ng EK, Tsui NB, Lau TK, Leung TN, Chiu RW, et al. (2003) mRNA of placental origin is readily detectable in maternal plasma. *Proc Natl Acad Sci USA* 100(8): 4748-4753.
- Tsui NB, Wong BC, Leung TY, Lau TK, Chiu RW, et al. (2009) Non-invasive prenatal detection of fetal trisomy 18 by RNA-SNP allelic ratio analysis using maternal plasma SERPINB2 mRNA: a feasibility study. *Prenat Diagn* 29(11): 1031-1037.
- Tsui NB, Akolekar R, Chiu RW, Chow KC, Leung TY, et al. (2010) 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* 56(1): 73-81.
- Pang WW, Tsui MH, Sahota D, Leung TY, Lau TK, et al. (2009) A strategy for identifying circulating placental RNA markers for fetal growth assessment. *Prenat Diagn* 29(5): 495-504.
- Fujito N, Samura O, Miharu N, Tanigawa M, Hyodo M, et al. (2006) Increased plasma mRNAs of placenta-specific 1 (PLAC1) and glial cellmissing1 (GCM1) in mothers with pre-eclampsia. *Hiroshima J Med Sci* 55(1): 9-15.
- Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, et al. (2007) Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nat Med* 13(2): 218-223.
- Dondorp W, de Wert G, Bombard Y, Bianchi DW, Bergmann C, et al. (2015) Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *Eur J Hum Genet* 23(11): 1592.
- Huang X, Zheng J, Chen M, Zhao Y, Zhang C, et al. (2014) Noninvasive prenatal testing of trisomies 21 and 18 by massively parallel sequencing of maternal plasma DNA in twin pregnancies. *Prenat Diagn* 34(4): 335-340.
- Taylor MJ, Fisk NM (2000) Prenatal diagnosis in multiple pregnancy. *Baillieres Best Pract Res Clin Obstet Gynaecol* 14(4): 663-675.
- Cleary-Goldman J, D'Alton ME, Berkowitz RL (2005) Prenatal diagnosis and multiple pregnancy. *Semin Perinatol* 29(5): 312-320.
- Audibert F, Gagnon A (2011) Prenatal screening for and diagnosis of aneuploidy in twin pregnancies. *J Obstet Gynaecol Can* 33(7): 754-767.
- Blumenfeld YJ, Momirova V, Rouse DJ, Caritis SN, Sciscione A, et al. (2014) Accuracy of sonographic chorionicity classification in twin gestations. *J Ultrasound Med* 33(12): 2187-2192.
- Summers AM, Langlois S, Wyatt P, Wilson RD (2007) Prenatal screening for fetal aneuploidy. *J Obstet Gynaecol Can* 29(2): 146-179.
- Sebire NJ, Souka A, Skentou H, Geerts L, Nicolaides KH (2000) Early prediction of severe twin-to-twin transfusion syndrome. *Hum Reprod* 15(9): 2008-2010.
- Bush MC, Malone FD (2005) Down syndrome screening in twins. *Clin Perinatol* 32(2): 373-386.
- Simpson LL (2013) Ultrasound in twins: dichorionic and monochorionic. *Semin Perinatol* 37(5): 348-358.
- Ippolito DL, Bergstrom JE, Lutgendorf MA, Flood-Nichols SK, Magann EF (2014) A systematic review of amniotic fluid assessments in twin pregnancies. *J Ultrasound Med* 33(8): 1353-1364.
- Suhag A, Berghella V (2015) Short Cervical Length Dilemma. *Obstet Gynecol Clin North Am* 42(2): 241-254.
- Muller F, Dreux S, Dupoizat H, Uzan S, Dubin MF, et al. (2001) Second-trimester Down syndrome maternal serum screening in twin pregnancies: impact of chorionicity. *Prenat Diagn* 23(4): 331-335.
- Cleary-Goldman J, Berkowitz R (2005) First trimester screening for Down syndrome in multiple pregnancy. *Semin Perinatol* 29(6): 395-400.
- Prats P, Rodríguez I, Comas C, Puerto B (2012) First trimester risk assessment for trisomy 21 in twin pregnancies combining nuchal translucency and first trimester biochemical markers. *Prenat Diagn* 32(10): 927-932.
- Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, et al. (2005) First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 353(19): 2001-2011.

31. Efrat Z, Perri T, Ramati E, Tugendreich D, Meizner I (2006) Fetal gender assignment by first-trimester ultrasound. *Ultrasound Obstet Gynecol* 27(6): 619-621.
32. Finning KM, Chitty LS (2008) Non-invasive fetal sex determination: impact on clinical practice. *Semin Fetal Neonatal Med* 13(2): 69-75.
33. Avent ND, Chitty LS (2006) Non-invasive diagnosis of fetal sex; utilisation of free fetal DNA in maternal plasma and ultrasound. *Prenat Diagn* 26(7): 598-603.
34. Mortarino M, Garagiola I, Lotta LA, Siboni SM, Semprini AE, et al. (2011) Non-invasive tool for fetal sex determination in early gestational age. *Haemophilia* 17(6): 952-956.
35. Orendi K, Klein K, Krampl-Bettelheim E, Nuk M, Holzapfel-Bauer M, et al. (2011) SRY-specific cell free fetal DNA in maternal plasma in twin pregnancies throughout gestation. *Placenta* 32(8): 611-615.
36. Attilakos G, Maddocks DG, Davies T, Hunt LP, Avent ND, et al. (2011) Quantification of free fetal DNA in multiple pregnancies and relationship with chorionicity. *Prenat Diagn* 31(10): 967-972.
37. Picchiassi E, Di Renzo GC, Tarquini F, Bini V, Centra M, et al. (2012) The potential usefulness of free fetal DNA in maternal blood for prenatal fetal gender determination in multiple pregnancies. *Twin Res Hum Genet* 15(2): 143-148.
38. Leung TY, Qu JZ, Liao GJ, Jiang P, Cheng YK, et al. (2013) Noninvasive twin zygosity assessment and aneuploidy detection by maternal plasma DNA sequencing. *Prenat Diagn* 33(7): 675-681.
39. Qu JZ, Leung TY, Jiang P, Liao GJ, Cheng YK, et al. (2013) Noninvasive prenatal determination of twin zygosity by maternal plasma DNA analysis. *Clin Chem* 59(2): 427-435.
40. Struble CA, Syngelaki A, Oliphant A, Song K, Nicolaides KH (2014) Fetal fraction estimate in twin pregnancies using directed cell-free DNA analysis. *Fetal Diagn Ther* 35(3): 199-203.
41. del Mar Gil M, Quezada MS, Bregant B, Syngelaki A, Nicolaides KH (2014) Cell-free DNA analysis for trisomy risk assessment in first-trimester twin pregnancies. *Fetal Diagn Ther* 35(3): 204-211.
42. Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, et al. (2011) Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cellfree fetal DNA from maternal blood. *Clin Chem* 57(7): 1042-1049.
43. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, et al. (2011) DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 13(11): 913-920.
44. Lau TK, Cheung SW, Lo PS, Pursley AN, Chan MK, et al. (2014) Non-invasive prenatal testing for fetal chromosomal abnormalities by low coverage whole genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center. *Ultrasound Obstet Gynecol* 43(3): 254-264.
45. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH (2013) Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol* 42(1): 34-40.
46. Mao J, Wang T, Wang BJ, Liu YH, Li H, et al. (2014) Confined placental origin of the circulating cell free fetal DNA revealed by a discordant non-invasive prenatal test result in a trisomy 18 pregnancy. *Clin Chim Acta* 433: 190-193.
47. Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH (2012) Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 206(4): 322.e1-322.e5.
48. Canick JA, Kloza EM, Lambert-Messerlian GM, Haddow JE, Ehrlich M, et al. (2012) DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn* 32(8): 730-734.
49. Lau TK, Jiang F, Chan MK, Zhang H, Lo PS, et al. (2013) Non-invasive prenatal screening of fetal Down syndrome by maternal plasma DNA sequencing in twin pregnancies. *J Matern Fetal Neonatal Med* 26(4): 434-437.
50. Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, et al. (2009) Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. *Am J Obstet Gynecol* 200(1): 98.e1-98.e6.
51. Lo YM, Chiu RW (2010) Noninvasive approaches to prenatal diagnosis of hemoglobinopathies using fetal DNA in maternal plasma. *Hematol Oncol Clin North Am* 24(6): 1179-1186.
52. Jakobsen TR, Clausen FB, Rode L, Dziegiel MH, Tabor A (2013) Identifying mild and severe preeclampsia in asymptomatic pregnant women by levels of cell-free fetal DNA. *Transfusion* 53(9): 1956-1964.
53. Wataganara T, Gratacos E, Jani J, Becker J, Lewi L, et al. (2005) Persistent elevation of cell-free fetal DNA levels in maternal plasma after selective laser coagulation of chorionic plate anastomoses in severe midgestational twin-twin transfusion syndrome. *Am J Obstet Gynecol* 192(2): 604-609.
54. Ge Q, Li H, Yang Q, Lu J, Tu J, Bai Y, et al. (2011) Sequencing circulating miRNA in maternal plasma with modified library preparation. *Clin Chim Acta* 412(21): 1989-1994.