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Amniocentesis, Chorionic Villi Sampling, Circulating Cell-Free Fetal DNA: A Comparative Study

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Abstract: *Non-invasive prenatal genetic testing (NIPT) can effectively use to detect circulating cell-free fetal DNA (cffDNA) in maternal peripheral blood which is a prenatal screening with great potential. The purpose of this study was to evaluate feasibility of NIPT by using Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) in maternal peripheral blood and compare with amniocentesis (AC) and chorionic villus sampling (CVS) methods for detection of chromosomal abnormalities. One hundred pregnant women aged between 15-46 years with 10-20 weeks of gestation after grouping by exclusion of criteria were enrolled in this study. Total abnormalities by amniocentesis were detected in 19 cases (21.11%). A total of 10 cases were evaluated by CVS that four of them were observed with one of the chromosomal abnormalities. Fetal DNA was extracted from the mother's blood and multiplex PCR reaction was performed using the "Aneufast QF-PCR" kit and the product was electrophoresed in "ABI Genetic Analyzer". Totally, four cases of Down syndrome, Edward syndrome, Turner syndrome, and triploidy were reported. The results of this study indicate that it is possible to detect most of the numerical chromosomal abnormalities by QF-PCR technique. The structural chromosomal abnormalities including chromosomal polymorphism 15 ps+, chromosomal polymorphism 14ps+, pericentric inversion of chromosome 9, robertsonian translocation rob (14;21) (q10; q10), chromosomal translocation t (2;10) (q10; q10), and unknown marker were not identified. Precise prenatal diagnosis of embryonic chromosomal abnormalities by AC and/or CVS are the most common indication of prenatal assays, because the cffDNA analysis alone cannot detect all possible chromosomal abnormalities.*

Keywords: *Cytogenetic Analysis, Amniocentesis, Chorionic Villi Sampling, Quantitative Fluorescence-Polymerase Chain Reaction*

INTRODUCTION

Non-invasive prenatal genetic testing (NIPT) can effectively use to detect circulating cell-free fetal DNA (cffDNA) in maternal peripheral blood which is a prenatal screening with great potential (Minear et al., 2015). A growing number of studies have confirmed that non-invasive fetal aneuploidy chromosomal testing achieved an accuracy of above 99% for trisomy 21 syndrome and trisomy 18 syndrome, as well as up to 91% for trisomy 13 syndrome (Zhang et al., 2016; Norton et al., 2012; Palomaki et al., 2011). Fetal aneuploidy and other chromosomal aberrations affect 9 out of 1000 live births, due to various mechanisms and advanced maternal age (Sherman et al., 1994; Petersen and Mikkelsen, 2000). A study on the factors influencing clinical application of NIPT showed that pregnant women chose NIPT because of less trauma, low risk, and high

safety to fetal (Skirton and Patch, 2013). However, a recent study found that although the accuracy of NIPT in disease diagnosis is still questionable, the number of pregnant women with positive genetic abnormalities who should receive further invasive genetic testing has decreased significantly with the popularization of NIPT (Li et al., 2016).

The first clues of circulating nucleic acids in the maternal peripheral blood was shown in 1948 (Mandel and Metals, 1948) and the presence of cffDNA in the plasma of pregnant women reported by Dennis Lo et al for the first time (Norton et al., 2012). Studies have shown that cffDNA is first seen in 4 weeks of gestation and cffDNA increases with progression of pregnancy (Lo et al., 1998). The discovery of cffDNA in maternal plasma completely altered non-invasive prenatal screening applications (Porreco et al., 2014). The cffDNA in the plasma typically has about 150-180 base pairs in length and most of them originated from apoptotic cells (Neufeld-Kaiser, Cheng and Liu, 2015). Particularly, cffDNA has its origin in the placental cytotrophoblastic cells, which are released into maternal bloodstream during pregnancy (Neufeld-Kaiser, Cheng and Liu, 2015) and usually accounts for approximately 10–20% of the average of cell-free DNA in the maternal plasma in the second trimester of gestation (Song et al., 2015).

In pregnant women who are diagnosed at high risk for such an aneuploidies by using biochemical and/or ultrasound screening tests, they should use invasive prenatal diagnostic tests such as chorionic villus sampling (CVS) and/or amniocentesis (AC). However, the use of these types of invasive diagnostic methods can consist of one percent risk of abortion. AC which has been applied since the end of the 19th century in single cases summarized by Milunsky (Milunsky, 1979), became the first routine genetic invasive prenatal test at the end of the 1960s as a tertiary procedure reserved for only the highest-risk patients (Jacobson and Barter, 1967; Wang and Cheng, 2009). Although this technique is familiar in clinical practice, the main role of AC is still the recognition of chromosomal abnormalities and recognized known clinical or inherited genetic determinations (Chen et al., 2010:341-50; Chen et al., 2010:455-67; Chen et al., 2011:48-57; Chen et al., 2011:245-8; Chen et al., 2011:390-3; Chen et al., 2011:331-8).

In addition, the acquisition of CVS was introduced as a routine approach in the 1980s; here the goal is to acquire genetic information of the developing child a few weeks earlier than is possible with AC (Ward RH et al., 1983), even though CVS performed with the goal to do genetic studies has been reported before (Mohr, 1968; Kullander and Sandahl, 1973; Hahnemann, 1974; No authors listed, 1975), it never became routine until the study of Ward et al. (1983). While chromosomes studied from CVS represent placenta-derived cells, umbilical cord blood sampling enables chromosomal studies in fetal cells (Gosden, Nicolaides and Rodeck, 1988).

The purpose of this study was to evaluate feasibility of non-invasive prenatal test by using QF-PCR in maternal peripheral blood and compare with AC and CVS methods for detection of chromosomal abnormalities. Our alternative hypothesis is that NIPT has gained a growing role in prenatal chromosomal abnormalities diagnosis.

Materials and Methods

The study was approved by the Committee of Ethics. One hundred pregnant women aged between 15-46 years (Mean=33.42 years and Median=35 years) with a 10-20 weeks of gestation (Mean=15.30 weeks and Median=15 weeks) who positive quad-marker test, referring to gynecologist of Alzahra and Taleghani hospitals in Tabriz province (north western of Iran), were enrolled in this study. The majority (90%) of the patients (n: 90) underwent AC and 10% (n: 10) CVS. Fifty percent of these patients were selected for non-invasive prenatal screening (NIPS) by some, detects defects in cffDNA after purified from the pregnant woman's blood.

Amniocentesis was performed using a freehand technique by a 22-gauge needle under continuous ultrasound guidance. Under the guidance of ultrasound, the angulation and direction of the needle were performed in the most appropriate place to observe amniotic fluid and fetus organs to avoid collision of needles with placenta

and umbilical cords. Depending on the circumstances, one of two transcervical or transabdominal approaches was used for CVS. Transcervical and transabdominal CVS were performed using a 16-gauge silver cannula with an 18 gauge guide needle and an aspiration needle of gauge 21, respectively.

Twenty milliliters of amniotic fluid and sample of chorionic villi were collected and transferred directly to the laboratory for culture. After centrifugation at 1600 rpm for 8 min, the supernatant was discarded, and 5 mL culture medium was inoculated with 1-1.5 mL cell suspension. The culture continued for 6 to 7 days at 37 ° C and 5% CO2 until cell growth was observed under inverted microscope. G-banding was used to prepare the chromosome samples, followed by ISCN 2009 for analysis of karyotypes.

We collected 5-10 ml of maternal peripheral blood into a tube containing EDTA. Blood samples were centrifuged at 3,000g, and plasma was carefully removed from the EDTA-containing tube, and were transferred into plain polypropylene tubes. Plasma samples were re-centrifuged at 3,000g, and supernatants were freshly in polypropylene tubes. Samples were stored at -20 ° C for further evaluation.

DNA Extraction from Plasma Samples

DNA was extracted from plasma samples using an EpiQuik Kit (Epi-GENTEK, USA). Genomic DNA was isolated from 200-µL of maternal blood sample using a High Pure PCR Template Preparation Kit (Roche, USA) according to the manufacturer's instructions. QF-PCR experiments were performed with a commercially available Devyser Complete QF-PCR kit Version 1 (Devyser, Sweden). At least 7 STR markers for each 3 autosomal (13, 18, 21) and 2 sex (X, Y) chromosomes were analyzed, and 50-ng/µL DNA samples were used per PCR mix. PCR was performed in 25-µL total volume. The PCR conditions were as follows: first denaturation at 95 °C for 15 min, 26 cycles for denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s, extension at 72°C for 90 s, and final extension at 72 °C for 30 min.

Eight microliters of PCR products were mixed with 10 µL of formamide and 0.5 µL of ROX size standard (ABI, USA) in a MicroAmp (Applied Biosystems, USA) optical 96-well reaction plate. After being denatured for 3 min at 95 °C and cooled for 3 min at –20 °C, capillary electrophoresis was performed in an ABI 3130 system (ABI, USA). The GeneScan Analysis program was used for determination of peak length and areas.

Results

The mean level of serum markers in the present study was 1.67, 1,87, 0.89, 0.96 and 1.01 for Inhibin A, ß-hCG, uE3, AFP, and PAPP-A, respectively. In this study, analysis was carried out on 90 amniotic fluid samples were examined for cytogenetics. Total abnormalities by amniocentesis were detected in 19 cases (21.11%) and are shown from highest to lowest: 4 cases (4.4%) were with Down syndrome, 3 (3.3%) cases with rare chromosomal polymorphism 15 ps^+, 3 (3.3%) cases with rare chromosomal polymorphism 22 ps^+, 3 (3.3%) cases with pericentric inversion of chromosome 9, 2 cases (2.2%) with rare chromosomal polymorphism 14ps^+, 1 (1.1%) case with Edward syndrome, 1 (1.1%) case with robertsonian translocation rob (14;21) (q10;q10), 1 (1.1%) case with chromosomal translocation t (2;10) (q10;q10), and 1 (1.1%) case with an unknown marker.

A total of 10 cases were evaluated by CVS that four of them were observed with one of the following chromosomal abnormalities including patau syndrome, Turner syndrome, triploidy, and trisomy 16 syndrome. Finally, fifty maternal blood samples were analyzed by QF-PCR technique. Fetal DNA was extracted from the mother's blood and multiplex PCR reaction was performed. Totally, Four cases of Down syndrome, Edward syndrome, Turner syndrome, and triploidy were reported.

Discussion

The results of this study indicate that it is possible to detect most of the numerical chromosomal abnormalities, such as trisomy 21 by QF-PCR technique. The structural chromosomal abnormalities including chromosomal polymorphism 15 ps^+, chromosomal polymorphism 14ps^+, pericentric inversion of chromosome 9, robertsonian translocation rob (14;21) (q10;q10), chromosomal translocation t (2;10) (q10;q10), and unknown

marker were not identified. Therefore, invasive methods such as amniocentesis should be still use. Precise prenatal diagnosis of embryonic chromosomal abnormalities by AC and/or CVS are the most common indication of prenatal assays, because the cffDNA analysis alone can not detect all possible chromosomal abnormalities. Today, in advanced countries, the use of screening methods for pregnant women is suggested to perform one of the perinatal diagnostic invasive tests, such as AC or CVS.

Torella and colleagues performed 713 singleton pregnancies screening between 8-16 weeks of pregnancy based on the levels of ß-hCG and PAPP-A, and in the second stage, 12-18 weeks, screening was performed based on NT thickness. Totally, 23 positive cases were reported, with 5 cases of trisomy 21. The 707 cases had normal karyotype, but 6 cases showed karyotype trisomy 21 syndrome (Torella et al., 2013).

A study on 7647 normal pregnancies between 14-21 weeks between 2007 and 2010 in China indicates that levels of AFP, uE3 are increasing and hCG is declining in the second trimester, when the maternal weight increased, these markers decreased. These results indicate that the information in the laboratory from these biochemical markers should be consistent with the information available from the local population (Wan et al., 2012). A similar study was performed on 661 amniotic fluid samples, which reported 59 cases (8.9%) of Down syndrome fetuses. The main difference between the study and our study was the failure to report other chromosomal abnormalities and the lack of use of CVS (Rijad Konjhodzic et al., 2014).

Wang et al reported a chromosomal abnormality of 4.6% (68 (2.72) and 41 (1.64) cases had numerical and structural abnormalities, respectively in a retrospective study of 2500 amniocentesis samples conducted between 2010 and 2014 at Jilin University (Porreco et al., 2014).

In a study of 1244 amniocentesis samples and 2556 CVS samples in 2002-2012, 4.2% chromosomal translocation was reported. In contrast to our study, there are no reports of numerical chromosomal abnormalities in this study (Skirton and Patch, 2013).

Rostami and colleagues in Iran conducted a study to diagnose early onset aneuploidy by QF-PCR compared with the results obtained from the cytogenetic method. The results of this study showed that QF-PCR technique was not able to detect numerical chromosomal abnormalities in comparison with cytogenetic method, which was similar in our study. This study also revealed the inability of the QF-PCR technique to detect sexual mosaicism (Rostami Parvin et al., 2011).

In a study, comparing the three methods of AC, CVS and cordocentesis with QF-PCR technique showed that the QF-PCR technique, in addition to the proper examination of different classical trisomy and monosomy, can also report the mosaicism, however, depending on that the mosaicism is equal to or above 23%. However, we were unable to declare mosaicism through the QF-PCR technique in our study (Porreco et al., 2014).

Sa'idi et al. in 2010 conducted a study on pregnant women at the Costa Rabba Hospital in India with a positive family history of chromosomal abnormalities and high age. All trisomy samples were double-checked by QF-PCR technique and it was observed that samples with abnormalities are confirmed by QF-PCR technique too. The difference between this study and our study was the use of amniotic fluid for QF-PCR testing, and because all the cases had only trisomy disorder, not other chromosomal abnormalities, therefore, the diagnostic weakness of the QF-PCR technique was not specified (Skirton and Patch, 2013).

Conclusion

The NIPT and use QF-PCR technique from peripheral blood of a pregnant woman, despite having positive points such as short-term response, have limitations and false-negative results for the most common aneuploidies, therefore, this test should only be considered as a screening test for some numerical chromosomal abnormalities, not as a definitive diagnostic test. For prenatal screening, you need to have a thorough genetic counseling and, if necessary, even use the invasive methods of detecting chromosomal abnormalities.

Conflict of Interest

The authors have declared no competing interests; none of the authors have any conflict of interest or relation with any third part that bias the publication of this report.

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