Research Article

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Rapid Prenatal Diagnosis of Chromosomal Aneuploidies Using Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR)

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Abstract

Introduction: Prenatal diagnosis plays an important role in determining the health of fetus, which is why rapid diagnostic tests are so important. One of these quick diagnostic tests is quantitative fluorescent-polymerase chain (QF-PCR) technique. This technique employed to detect chromosomal aneuploidies (13, 18, 21, X and Y). In this method, there is no need for cell culturing. QF-PCR is rapid, suitable for automation, low cost and faster turnaround time.

Material and Methods: In this study, 260 patients were studied, and analyzed by 26 short tandem repeat (STR) markers for detection of chromosomal abnormalities. The QF-PCR assay was performed by Devyser kit (Sweden). First DNA was extracted, and then PCR was performed, PCR products were run on capillary electrophoresis system, and finally analyzed by the Genemarker Software.

Results: The results of QF-PCR were as follows: trisomy 21 (6 cases), 18 (3 cases), triploidy (1 case), klinefelter syndrome (1 case), without suspicion of any mosaicism.

Conclusion: Prenatal diagnostic outcomes give early results, thus reducing parental anxiety and increasing clinical management of a high risk pregnancy.

Keywords: QF-PCR, Aneuploidy, Prenatal diagnosis, Trisomy, Short Tandem Repeats.

Introduction

Quantitative fluorescent polymerase chain reaction (QF-PCR) technique is used for rapid prenatal diagnosis of common Chromosomal aneuploidies in many laboratories around the world [1]. This technique is widely applied for the analysis of heterozygosities of markers (microsatellites), short tandem repeat (STR), on autosomes chromosomes 13 (Patau syndrome), 18 (Edward syndrome), 21 (Down syndrome), and sex chromosomes; X and Y such as Turner syndrome and Klinefelter) [2]. After amplification by fluorescent labelled primers, microsatellite alleles copy number and allele peak is measured [3].

Amniotic fluids and chorionic villi used for QF-PCR, without prior culturing of fetal cells [4]. This method had been introduced for more than 20 years (since 1993), then as an NHS diagnostic test in 2000 and is now improved and widely used technique [3,5,6]. With this preparation turnaround times decreased and anytherapeutic measurements can be applied only after 2-3 days [7]. Other advantages are; Affordable cost, reliable aneuploidy detection, high sensitivity and high specificity for the diagnosis of aneuploidies of chromosomes X, Y, 13, 18 and 21 [8,9]. An extra copy or loss of one chromosome, either in one or all cells cause common chromosome anomalies [10]. Chromosomal changes can be categorized into two general groups: Changes that affect the structure (structural changes in chromosomes) of the chromosome and changes that affect the number of chromosomes (numerical changes in chromosomes).

Due to the large extent of the change, numerical changes have more destructive effects. An aneuploidy is a component of numerical changes in chromosomes [11]. Aneuploidies are important because they cause perinatal morbidity and mortality and childhood handicap [12]. Diagnosis of aneuploidy in the 18 to 19 weeks
of pregnancy is important for managing high risk pregnancy. Of course, early diagnosis can be done at lower weeks of pregnancy by karyotyping of CVS, which requires cell culture that is time-consuming and the time required to do cytogenetic analysis is a disadvantage of karyotyping [3]. There are number of syndromes attributed to chromosomal aneuploidies, which are severe mental retardation, multiple dysmorphic features, growth retardation, growth and developmental delay [3,13].

The first trimester screening tests are ultrasound and biochemical screening from maternal blood which are non-invasive techniques [14]. Indications for prenatal invasive diagnosis are: positive biochemical screen advanced maternal age, positive maternal serum screening result, ultrasound abnormality, family history of a chromosome or single gene disorder, and etc. [5,14]. Later invasive testing were considered for high risk patients [12]. An invasive method involves needles being inserted into the uterus, e.g. amniocentesis, which can be done from about 14 weeks gestation, and usually up to about 20 weeks, and chorionic villus sampling, which can be done earlier (between 9.5 and 12.5 weeks gestation) but which may be slightly more risky to the fetus. Screening approval is important and invasive testing is recommended for those at high risk. This will increases the diagnosis of abnormal fetuses especially Down syndrome [14].

The aim of this study is determining the importance of QF-PCR method and recognize referral cause in prenatal diagnosis.

Materials and Methods
Sample preparation
We performed QF-PCR on a total of 260 prenatal amnion samples. Genomic DNA was isolated after centrifugation of 5 ml sample, at 17000 rpm for 10 minutes (Eppendorf 5415-R). The pellets were cleared by washing with 200μl 1×PBS (phosphate-buffered saline, pH 7.4) buffer. DNA was extracted using AmpliSens, Russiakit according to the manufacturer’s instructions. Multiplex QF-PCR assays were set up (Devyser, Sweden) for detection of STRs. The Devyser QF-PCR Kit contains 26 multiplex marker of short tandem repeats (STR) which are five STRs from chromosomes 13 (D13S742, D13S634, D13S628, D13S305, D13S1492), five from chromosome 18 (D18S978, D18S535, D18S386, D18S976, GATA178F11), six from chromosome 21 (D21S1435, D21S11, D21S1411, D21S1444, D21S1442, D21S1437), and ten STRs from chromosome X and Y (DXS1187, XHPRT, DXS2390, SRY, DXYS267, DXYS218, AMELX, AMELY, ZFY, ZFX).

STR list of marker locations and labeling information for the Deyser kit was given in (Table 1).

| ID | Location | Marker | Marker size range (bp) | Dye Colour |
|----|----------|--------|------------------------|------------|
| 13A | 13q12.12 | D13S742 | 222-334 | Green |
| 13B | 13q21.32-q21.33 | D13S634 | 365-435 | Blue |
| 13C | 13q31.1 | D13S628 | 420-475 | Yellow |
| 13D | 13q13.3 | D13S305 | 435-505 | Green |
| 13K | 13q21.1 | D13S1492 | 100-175 | Red |
| 18B | 18q12.3 | D18S978 | 195-230 | Yellow |
| 18C | 18q12.3 | D18S535 | 300-350 | Blue |
| 18D | 18q22.1 | D18q22.1 | 338-430 | Green |
| 18J | 18p11.31 | D18S976 | 440-495 | Red |
| 18M | 18p11.32 | GATA178F11 | 350-410 | Yellow |
| 21A | 21q21.3 | D21S1435 | 150-208 | Blue |
| 21B | 21q21.1 | D21S11 | 215-290 | Blue |
| 21C | 21q22.3 | D21S1411 | 245-345 | Yellow |
| 21D | 21q22.13 | D21S1444 | 440-495 | Blue |
| 21H | 21q21.3 | D21S1442 | 362-420 | Red |
| 21I | 21q21.1 | D21S1437 | 105-152 | Yellow |
| X1 | DXS1187 | Xq26.2 | 120-170 | Green |
| X3 | XHPRT | Xq26.2-q26.3 | 265-308 | Red |
| X9 | DXS2390 | Xq27.1-q27.2 | 312-357 | Red |
| SRY | SRY | Yp11.31 | 236 | Yellow |
| XY2 | DXYS267 | Xq21.31,Yp11.31 | 175-217 | Green |
| XY3 | DXYS218 | Xp22.33, Yp11.32 | 215-260 | Red |
| AMELXY | AMELX, AMELY | Xp22.2, Yp11.2 | X=104, Y=110 | Blue |
| ZFYX | ZFY, ZFX | Yp11.31, Xp22.11 | 157-166 | Yellow |
| T1 | ZFY, ZFX | Yp11.31, Xp22.11 | 7=181, X=201 | Red |
| T3 | - | 3p24.2, Xq21.1 | 3=133, X=137 | Blue |
Thermal Cycler Program for amplification was as follows: Initial denaturation at 95 °C for 15 min, 94 °C 30 sec; 58 °C 1 min 30 sec; 72 °C 1 min 30 sec for 27 cycles, and final extension at 72 °C for 30 min.

**Fragment and data analyses**
Analysis of PCR product from fragment was done by ABI 3130 XL capillary electrophoresis on pop7 polymer. Finally, data were analyzed by Genemarker Software.

**Results**
Among the study population, 105 samples showed normal male pattern (46, XY), 144 showed normal female pattern (46,XX), 3 samples showed male pattern with trisomy 21 (47 XY +21), 3 samples showed female pattern with trisomy 21 (47 XX +21), 3 samples showed female pattern with trisomy 18 (47XX +18), 1 sample showed triploid female pattern, and 1 sample showed Klinefelter syndrome 47, XXY.

The largest abnormal result was Trisomy 21 of abnormal results. The results of the 260 specimens are presented in (Table 2).

| Results | QF-PCR Results |
|---------|---------------|
| Trisomy18 | 3 |
| Trisomy21 | 6 |
| Triploidy | 1 |
| Klinefelter syndrome | 1 |
| Total | 260 |

95.7 % of pregnant women were healthy (Table 3). Only one sample of all specimens was detected of Maternal cell contamination (MCC) (Table 3).

| Indications | Number | Percent |
|-------------|--------|---------|
| Normal | 249 | 95.7 |
| Abnormality | 11 | 4.2 |
| MCC | 1 | 0.3 |
| Total | 260 | 100 |

Indications for testing with QF-PCR (n=260) were as follows (Table 4) out of 260 samples, 96 cases were of positive biochemical screen for maternal serum, 15 cases of advanced maternal age, 10 cases of ultrasound abnormality, 32 cases of other indications. Some cases had 2 common reason for referral, advanced maternal age and abnormal maternal serum biochemical screening (93 case) and the number with just abnormal maternal serum biochemical screening was 14 cases.

| Indications | No. of patients |
|-------------|----------------|
| Positive biochemical screen (a maternal serum screening result) | 96 |
| AMA (advanced maternal age) | 15 |
| U/S abn (ultrasound abnormality) | 10 |
| Other | 32 |
| Positive biochemical screen and AMA | 93 |
| 93 | 14 |

Results show increased risk for Down syndrome and other syndromes in “older” pregnant women (Table 5). Table 5 shows pregnant women aged 41-45 are more exposed to dangers, especially Down syndrome.

| Maternal age(years) | No. of patients | Trisomy 21 | Trisomy 18 | Klinefelter | Triploidy | Total |
|---------------------|----------------|------------|------------|-------------|-----------|-------|
| <20                 | 0              | 0          | 0          | 0           | 0         | 0     |
| 21-25               | 0              | 1          | 0          | 0           | 1         | 2     |
| 26-30               | 0              | 0          | 0          | 0           | 0         | 0     |
| 31-35               | 1              | 0          | 0          | 1           | 2         | 4     |
| 36-40               | 1              | 0          | 0          | 0           | 1         | 2     |
| 41-45               | 4              | 2          | 1          | 0           | 7         | 11    |
| Total               | 6              | 3          | 1          | 1           | 11        | 26    |

For each allele, the height ratio indicating normal peak is between 0.8 and 1.4 and the area below each peak is at a 1:1 ratio (The QF-PCR peak profile samples of a fetus with normal male and normal female in Fig 1 and Fig 2). Higher than 1.8 or less than 0.8 considered as abnormal. Three peaks with a 1:1:1 ratio (trisomic triallelic subjects) or two peaks with a 2:1 ratio (trisomic diallelic subjects) are showing trisomy.

The QF-PCR peak profile samples of a fetus with trisomy 21 was given in Fig3. Homozygote markers are not informative and so they are not considered. However, if the number of homozygote markers increases, it should be also confirmed by another method, such as; full karyotype or FISH for confirmation. Non informative peak as an example is shown in Fig 3. In general, at least two markers that are heterozygotes are required for final confirmation of normality or abnormality (informative peaks shown in Fig 1, 2, 3) [2,6,10,15,16].
Discussion

There are several molecular techniques for diagnosis of aneuploidy, including: fluorescence in situ hybridization (FISH), Multiplex ligation-dependent probe amplification (MLPA), loss of heterozygosity (LOH) assays, microarray technology, fluorescence polymerase chain reaction (QF-PCR) and array comparative genomic hybridization (CGH) that each of which has disadvantages and advantages [1,17]. In this study, 260 amniotic fluid samples for the aneuploidy of chromosomes 13, 18, 21, X and Y were analyzed by QF-PCR. According to the results obtained, chromosomal status of chromosomes 13, 18, 21, X and Y were correctly detected by QF-PCR test. Neither false negativnor false positive samples were found in the results [18]. QF-PCR is one of the precise, rapid tests for detection of aneuploidy [19]. This method decreases cost of prenatal studies, workload in cytogenetic laboratories, faster turnaround time and avoidance of ambiguous karyotype results [8,18]. The data set presented here and other previous studies showed the accuracy, robustness, reliability, rapidness and success of QF-PCR method in detection of prenatal aneuploidy [10,15,19]. These are the merits of this test. This conclusion is a crucial factor in determining the fate of the fetus and the life of parents.

There are also limits for QF-PCR as it is unable to detect inversions, deletions, translocations, marker chromosomes and mosaicism [8,9]. Of course structural chromosomal abnormalities are rare and these abnormalities will change phenotypes of fetus that can often be detected with ultrasonography. Despite, QF-PCR can identify MCC and has better detection than interphase FISH and traditional karyotyping [7]. The greater the number of highly polymorphic STR markers examined the greater the diagnostic accuracy. Target chromosomal location of each marker is also important in the accuracy of results [8,20]. In this study 26 markers were investigated.

The use of prenatal rapid technique such as QF-PCR alone as diagnostic methods is still under discussion [8]. However, some studies suggest that QF-PCR, along with ultrasound examinations, detects at least 95% of chromosome anomalies. There is also a high level of coordination between the results of QF-PCR test and the karyotype [18]. Rapid diagnostic methods before childbirth, especially the QF-PCR test, help to alleviate stress in parents and allow family members to review decisions on the termination of pregnancy if there is an abnormality [10]. Our suggestion is to use both techniques together in order to use advantages of both techniques.

References

1. Sung-Hee Han, Jae-Song Ryu, Jeong-Wook An, M.T.Ok-Kyoung Park, Hye-Ryoung Yoon, et al. (2010) Validation of QF-PCR for Rapid Prenatal Diagnosis of Common Chromosomal Aneuploidies in Korea. Journal of Genetic Medicine 7: 59-66.
2. Kathy Mann, Celia Donaghue, Susan P Fox1, Zoe Docherty, Caroline Mackie Ogilvie (2004) Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. European Journal of Human Genetics 12: 907-915.
3. Najmeh Ahangari, Mohammad Doosti, Elaheh Ahangari, Nafise Baradaran Rafiei, Ehsan Ghayoor Karimian (2016) Prenatal diagnosis of fetal aneuploidies using g QF-PCR in 333 cases. Molecular Medicine Journal 2: 38-42.
4. You Jung Shin, Jin Hoon Chung, Do Jin Kim, Hyun Mee Ryu, Moon Young Kim, et al. (2016) Quantitative fluorescent polymerase chain reaction for rapid prenatal diagnosis of fetal aneuploidies in chorionic villus sampling in a single institution. Obstet Gynecol Sci59:444-453.
5. Özge Özker Kayal, Altuğ Koç, Taha Reşid Özdemir, Özgür Kirbiyik, Berk Özyılmaz, et al. (2017) QF-PCR in invasive prenatal diagnosis: a single-center experience in Turkey. Turk J Med Sci 47: 142-147.
6. Alison Hills, Celia Donaghue, Jonathan Waters, Katie Waters, Caroline Sullivan, et al. (2010) QF-PCR as a stand-alone test for prenatal samples: the first 2 years’ experience in the London region. Prenatal Diagnosis Prenat Diagn, Published online in Wiley Inter Science 30: 509-517.
7. Baig S, Ho SSY, Ng BL, Chiu L, Koay ESC, et al. (2010) Development of quantitative-fluorescence polymerase chain reaction for the rapid prenatal diagnosis of common chromosomal aneuploidies in 1,000 samples in Singapore. Singapore Med J 51: 343-348.
8. Celia Badenas, Laia Rodriguez-Revenga, Carme Morales, Carmen Mediano, Alberto Plaja, et al. (2010) Assessment of QF-PCR as the First Approach in Prenatal Diagnosis. Journal of Molecular Diagnostics 12: 828-834.
9. Sylvie Langlois, Vancouver BC, Alessandra Duncan, Montreal QC (2011) Use of a DNA Method, QF-PCR, in the Prenatal Diagnosis of Fetal Aneuploidies. JOGOC 265.
10. Akin Tekcan, Sengül Tural, Mehmet Elbistan, Nurten Kara, Davut Guven, et al. (2014) The combined QF-PCR and cytogenetic approach in prenatal diagnosis. Mol Biol Rep 41: 7431-7436.
11. Abdul Vahab Saadi, Pralhad Kushhtagi, PM Gopinath, Kapauu Satyamoorthy (2010) Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) for Prenatal Diagnosis of Chromosomal Aneuploidies. Int J Hum Genet 10: 121-129.
12. Madhusudan, Dey, Sumedha Sharma, Sumita Aggarwal (2013) Prenatal Screening Methods For Aneuploidies. N Am J Med Sci 5: 182-190.
13. Xiangdong Kong, Lin Li, Lei Sun, Kepeng Fu, Ju Long, et al. (2014) Rapid Diagnosis of Aneuploidy Using Segmental Duplication Quantitative Fluorescent PCR. March Volume 9: e88932.
14. Daniela Neagos, Ruxandra Cretu, Roxana Corina Sfetea, Laurentiu Camil Bohiltea (2011) The Importance of Screening and Prenatal Diagnosis in the Identification of the Numerical Chromosomal Abnormalities. A Journal of Clinical Medicine 6: 179-184.
15. Guzel Al1, Yilmaz MB, Demirhan O, Pazarbasi A, Kocaturk-Sel S, et al. (2012) Rapid Detection Of Fetal Aneuploidies By Quantitative Fluorescent Polymerase Chain Reaction For Prenatal Diagnosis In The Turkish Population. BJMG 15: 11-17.
16. Devyser User’s Manual (2017) Multiplex QF-PCR Kit. V2. Molgentix, Barcelona, pp 1-38.
17. You Jung Shin, Jin Hoon Chung, Do Jin Kim, Hyun Mee Ryu, Moon Young Kim, et al. (2016) Quantitative fluorescent polymerase chain reaction for rapid prenatal diagnosis of fetal aneuploidies in chorionic villus sampling in a single institution. Obstet Gynecol Sci 59: 444-453.
18. Diane J. Allingham-Hawkins, David Chitayat, Vincenzo Cirigliano, Anne Summers, Jason Tokunaga, et al. (2011) Prospective validation of quantitative fluorescent polymerase chain reaction for rapid detection of common aneuploidies. Genetics IN Medicine 13: 140-147.
19. Ioannis Papaoulidis1, Elisavet Siomou, Alexandros Sotiридis, George Efstathiou1, Anastasia Psara, et al. (2012) Dual testing with QF-PCR and karyotype analysis for prenatal diagnosis of
chromosomal abnormalities. Evaluation of 13500 cases with consideration of using QF-PCR as a stand-alone test according to referral indications. Prenatal Diagnosis 32: 680-685.
20. Habib Nasiri, Mohammad-Reza Noori-Dalooi, Jila Dastan, Saeed-Reza Ghaffari (2011) Investigation of QF-PCR Application for Rapid Prenatal Diagnosis of Chromosomal Aneuploidies in Iranian Population. Iran J Pediatr 21: 15-20.