Research Article

Journal of Gynecology & Reproductive Medicine

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

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Submitted: 26 Aug 2017; **Accepted**: 04 Sep 2017; **Published**: 08 Sep 2017

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 analysisof 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 measurementscan 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 whichare 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 atotal 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 extractedusing 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 markerof 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 Deyserkitwas given in **(Table 1)**.

Table 1: STR locations and labeling information for the Deyser kit

ID	Location	Marker	Marker size range (bp)2	Dye Colour
13A	13q12.12	D13S742	222 334	Green
13B	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	D 18q22.1	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	-	7q34, Xq13	7=181, X=201	Red
T3	-	3p24.2, Xq21.1	3=133, X=137	Blue

Thermal Cycler Program for amplification wasas 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 showedKlinefelter syndrome 47, XXY.

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

Table 2: Results of QF-PCR after fragment analysis

Results	QF-PCR Results
Trisomy18	3
Trisomy21	6
Triploidy	1
kelinfelter 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)**.

Table 3: The overall result of pregnant women with healthy and unhealthy embryo

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

Indications fortesting 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.

Table 4: Referral indications to run QF-PCR

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** showspregnant women aged 41-45 are more exposed to dangers, especially Down syndrome.

Table: 5 The distribution of known aneuploidies detected by QF-PCR as a function of maternal age

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

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 asabnormal. 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 arenot 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].

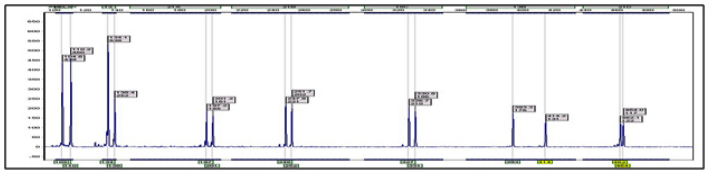

Figure 1: The QF-PCR peak profile samples of a fetus with normal male

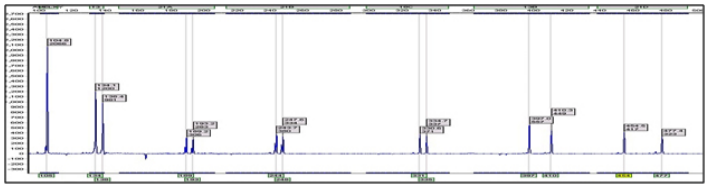

Figure 2: The QF-PCR peak profile samples of a fetus with normal female

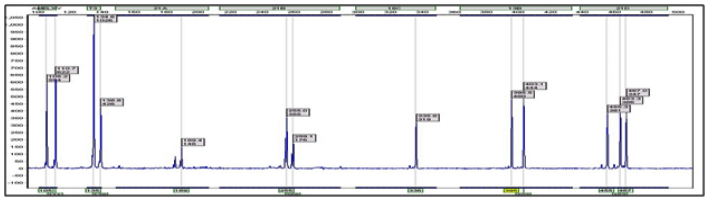

Figure 3: The QF-PCR peak profile samples of a fetus with trisomy 21

Discussion

There are several moleculartechniques for diagnosisof 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 negativenor 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 ambiguouskaryotype 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 that in order to use advantages of both techniques.

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