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Serpil AYDOĞMUŞ[1]
Hüseyin Levent KESKİN[1]
Semra BAYKAL GÖKÇE[2]
Ayşe Filiz AVŞAR[1]
Emine ÇELEN[1]

The efficacy of quantitative fluorescent-polymerase chain reaction (QF-PCR) in the diagnosis of prenatal aneuploidy

Aim: To investigate the efficacy of quantitative fluorescent-polymerase chain reaction (QF-PCR) in the diagnosis of aneuploidy.

Materials and methods: The study included 40 pregnant women considered to be at high risk, based on positive trisomy 21 screening results, that underwent amniocentesis for karyotyping to detect chromosomal anomalies. In amniotic fluid aneuploidy detection was carried out by both standard karyotyping and the QF-PCR method, and the results obtained with both methods were compared. In order to determine the efficacy of QF-PCR, its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Results: Of the 40 patients' results obtained with the 2 methods, 32 were similar. In 31 of these 32 cases, chromosome analysis interpreted as normal by QF-PCR was also established to be normal by standard karyotype analysis and 1 case evaluated as trisomy by QF-PCR was also determined to be trisomy 21 by standard karyotype analysis. In 6 cases, however, results were evaluated as chromosomal abnormality by QF-PCR, whereas standard analysis found them to be normal. The efficacy indices of QF-PCR were as follows: sensitivity 50%, specificity 83.7%, PPV 14.3%, and NPV 96.9%.

Conclusion: Until the efficacy of QF-PCR increases, along with the sophistication of the technique, conventional karyotype analysis will remain the gold standard.

Key words: Aneuploidy, QF-PCR conventional karyotype analysis

[1] Department of Obstetrics & Gynecology, Atatürk Education and Research Hospital
Ankara - TURKEY

[2] Department of Biochemistry & Clinical Biochemistry, Atatürk Education and Research Hospital,
Ankara - TURKEY

Prenatal anöploidi saptanmasında kantitatif floresan-polimeraz zincir reaksiyonu (QF-PCR) yönteminin etkinliği

Amaç: Bu çalışmanın amacı, uygun prenatal dönemde anöploidi tanısında kullanılan Kantitatif Floresan-Polimeraz Zincir Reaksiyonu (QF-PCR) yönteminin etkinliğinin araştırılmasıdır.

Yöntem ve gereçler: Trizomi 21 taraması sonucu pozitif olan ve bu nedenle yüksek riskli grup olarak kabul edildikten sonra kromozomal anomali araştırılması amacıyla amniosentez yapılan 40 gebe çalışmaya alındı. Amnion sıvısında hem standart karyotipleme ile kromozom analizi yapıldı, hem de QF-PCR metodu ile anöploidi incelemesi yapıldı. Her iki metod ile elde edilen sonuçlar karşılaştırıldı ve QF-PCR yönteminin etkinliğinin ortaya konması için sensitivite, spesifisite, pozitif ve negatif prediktif değerler (PPV, NPV) hesaplandı.

Bulgular: Her iki yöntemin sonuçları 40 olgudan 32'sinde benzerdi. Bu 32 olgudan 31'inde, QF-PCR yönteminde normal olarak değerlendirilen olguların standart karyotip analizinde normal karyotipe sahip olduğu kesinleştirildi ve QF-PCR yöntemi sonucu Trizomi 21 olarak değerlendirilen 1 olguda da standart karyotipleme sonucu Trizomi 21 olarak rapor edildi. 6 olguda ise QF-PCR sonuçları anormal olarak değerlendirilirken standart analizde bu olguların normal oldukları görüldü. QF-PCR'ın etkinlik göstergeleri, sensitivite % 50, spesifisite % 83,7, PPV % 14,3 ve NPV % 96,9 idi.

Sonuç: Teknikteki gelişme ile birlikte QF-PCR metodunun etkinliği artana kadar konvansiyonel karyotip analizi altın standart olarak kalmaya devam etmektedir.

Anahtar sözcükler: Anöploidi, QF-PCR, konvansiyonel karyotip analizi

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Correspondence
H. Levent KESKİN
Birlik Mah. 130 Sok. 3/5
06610 Çankaya,
Ankara - TURKEY

hlkeskin@yahoo.com

Introduction

Pregnant women with a high risk of carrying fetuses with chromosomal anomalies due to maternal age, abnormal serum biochemical markers, or abnormal ultrasonography findings, are evaluated by standard fetal karyotyping of fetal cells obtained from amniotic fluid or fetal blood via amniocentesis, chorionic villus sampling (CVS), or cordocentesis, which are all invasive methods. These conventional cytogenetic analyses remain the gold standard in the prenatal diagnosis of chromosomal anomalies (1).

Of these methods, the most frequently used for more than 25 years is the karyotyping of cells obtained from amniotic fluid; however, as the growth of cell cultures takes at least 10 days (optimum: 21 days), waiting for the results creates anxiety, for both the families and physicians.

Recently, various diagnostic methods that yield results in 24-48 h have been developed so that families and physicians are not subjected to an anxious waiting process. FISH (fluorescence in situ hybridization), QF-PCR (quantitative fluorescent-polymerase chain reaction), CGH (comparative genomic hybridization), and MLPA (multiplex ligation-dependent probe amplification) are the most frequently used of these methods (1-5). The aim of the present study was to compare the efficacy of QF-PCR, which yields results in 24-48 h, and standard karyotyping in the detection of chromosomal anomalies.

Materials and methods

Of the pregnant women that presented to Atatürk Education and Research Hospital, Obstetrics and Gynecology Department Antenatal Unit between June 2006 and April 2007, 40 pregnant women that underwent amniocentesis between the 16th and 20th gestational week (after consent for invasive intervention was obtained from the women and their spouses) due to positive screening results for trisomy 21 (those with a combined risk over 1/250) on one of the double or triple screening tests were included in the study. Amniotic fluid samples (20 mL) were obtained via amniocentesis guided by ultrasonography; 5 mL was used for QF-PCR and 15 mL was used for conventional cytogenetic analysis. Conventional cytogenetic analysis was performed by a private genetic laboratory. QF-PCR analysis was carried out in our laboratory with an ABI PRISM 310 device, using a multiplex QF-PCR rapid diagnosis kit for trisomy 21, 18, and 13, and sex chromosome aneuploidies (Aneufast™ QF-PCR). The Aneufast™ QF-PCR kit contains 6 multiplex markers (2 of them [S1, S2] for performing initial aneuploidy screening in a single electrophoresis, and 4 of them [M21, M13, M18, and MXY] chromosome-specific markers) and sets of short tandem repeats (STRs) that can be used for amplification of selected microsatellites and the amelogenin-SRY (sex determining region) (genes on the Y chromosome). This combination of markers allows the detection of aneuploidies involving chromosomes X, Y, 21, 18, and 13 for non-mosaic trisomies. This method is intended to be used to amplify DNA extracted from fresh prenatal samples, such as amniotic fluid, chorionic villus samples, or fetal blood. The Aneufast™ QF-PCR Kit uses a 5-dye fluorescent system for automated DNA fragment analysis. This allows multiplex amplification and electrophoresis of loci. In normal individuals heterozygous for the STRs, the same amount of fluorescence is generated for both alleles; therefore, the ratio between the area (and height) of the fluorescent peaks is 1:1. In homozygous individuals STR alleles have the same repeat number and size; therefore, quantification is not possible and the marker is uninformative. In a trisomic sample the 3 copies of a chromosome can be detected, as the corresponding chromosome-specific STRs show 3 peaks with the same fluorescent intensity and a ratio between the areas of 1:1:1 (trisomic triallelic). If 2 chromosomes have the same repeat number, quantitative PCR will produce 2 unbalanced fluorescent peaks with an area ratio of 2:1 (trisomic diallelic). Triploid samples will produce trisomic diallelic and triallelic patterns for informative STRs on all chromosomes. Due to the occasional preferential amplification of the smaller allele, the ratios between fluorescent peaks may vary within limits; ≤ 0.6-≥ 1.8:1 are assessed as trisomy.

The results of the QF-PCR rapid detection test were analyzed and interpreted by the author (S.B.G.), with the help of the geneticist of the private laboratory.

In each case, standard karyotype and QF-PCR results were compared in order to determine the efficacy of QF-PCR; its sensitivity, specificity, and positive and negative predictive values (PPV, NPV) were calculated.

Results

Mean age of the patients was 30.9 ± 7.1 years, mean number of gravidity was 2.9 ± 1.5, mean number of parity was 1.2 ± 0.7, and mean gestational age was 16 weeks + 6 days ± 1 week + 1 day. Of the 40 patients included in the study, the results of the 2 methods were similar in 32 (Table). In 31 of these cases chromosome analysis that was evaluated as normal by QF-PCR was also found to be normal by conventional karyotype analysis, and 1 case evaluated as aneuploidy (trisomy 21) by QF-PCR was interpreted in the same way by karyotype analysis. In 6 cases, although results were interpreted as aneuploidic anomaly in 13, 18, 21, and sex chromosomes by QF-PCR, they were found to be normal by karyotype analysis (false positive). Conversely, in 1 case QF-PCR interpreted the results as normal and conventional karyotype analysis determined the presence of 47, XYY chromosome (false negative). In 34 cases the results were obtained 24 h after the samples arrived at the laboratory. In 4 cases QF-PCR results were obtained in 48 h and in 1 case in 72 h because the analysis had to be repeated. In 1 patient interpretation could not be made because the amniotic fluid was hemorrhagic. The efficacy indexes of QF-PCR were as follows: sensitivity 50%, specificity 83.7%, PPV 14.3%, and NPV 96.9%.

Discussion

Trisomy in 13, 18, 21, and sex chromosomes constitute 60%-80% of abnormalities detected in amniotic fluid cultures (2). The stage of the cell culture, which lasts for 10-21 days before karyotype analysis, delays the process. Yet, with this method, the failure rate or the rate of negative cultures is lower than 0.5% (3).

The examination of amniotic fluid or CVS samples that are not cultured by molecular methods yields results much more rapidly, i.e. in 24-48 h. It is possible to detect aneuploidies of chromosomes 13, 18, and 21, and sex chromosomes by these methods.

QF-PCR, a rapid method for the detection of chromosomal aneuploidies in uncultured amniocytes, is a molecular screening method in which certain short tandem repeats on known chromosome loci are amplified by polymerase chain reaction. The test, thus, does not analyze chromosomes; only some markers are amplified to evaluate the number of copies of certain chromosomes, such as 13, 18, 21, X, and Y. A conventional karyotype analysis reveals the structure and number of all chromosomes, and detection sensitivity depends on the number of bands on the chromosomes. Nevertheless, these methods miss 15%-30% of anomalies detected by conventional karyotyping (4-9).

The FISH technique was approved by the FDA in 1997. It has been reported that the success rate of FISH and QF-PCR techniques in detecting trisomies of chromosome 13, 18, and 21 in those without mosaicism is around 70%-80% (2,4). Yılmaz et al. reported that the results of conventional cytogenetic and FISH methods were compatible in 48 out of 51 patients (5). In a comprehensive study carried out by Caine et al. in 2005, samples from 119,528 patients that underwent amniocentesis were examined and abnormal karyotypes were detected in 3081, and among those 2075 were examined using rapid aneuploid diagnosis methods (PCR and FISH); however, these methods were not successful in 1006 patients. Their sensitivity was hence reported to be 67% and the authors concluded that none of the 3 technologies—full karyotyping, PCR, and FISH—was completely reliable for detecting an abnormal karyotype, but that the best protocol for an interpretable result was to use PCR and karyotyping, or FISH and karyotyping, and that replacement of full karyotyping with rapid testing for trisomies 13, 18, and 21 after a positive screening for Down's syndrome will result in a substantial number of live born children with hitherto preventable mental or physical handicaps, and represents a substantial change in the outcome quality of prenatal testing offered to couples (4). In a study conducted by Mann et al. in 2001, QF-PCR yielded no false positive or false negative results in 1148 amniotic fluid samples and 188 CVS samples. Its sensitivity was reported to be 96.8%, its specificity 99.8%, and its PPV 96.7% (10). These tests have low success rates in the detection of other structural chromosomes and sex chromosome anomalies. Their most marked drawback is that they are unable to define small segment imbalances. Additionally, they cannot establish ring chromosomes or unbalanced translocations (3).

Table. Indications and results in the patients.

Case no.	Age	G	P	Indication results	Pregnancy week	Cytogenetic results	QF-PCR
1	31	2	1	TST/ DS	17 +3/7	46, XX	N, XX
2	27	3	2	TST/ DS	16 +3/7	46, XY	N, XY
3	36	2	1	DST/ DS	16	46, XX	N, XX
4	35	3	2	TST/ DS	18 +1/7	46, XX	N, XX
5	40	6	2	TST/ DS	17	46, XX	N, XX
6	38	3	2	TST/ DS	20	46, XY	N, XY
7	31	4	1	TST/ DS	18 +2/7	46, XY	N, XY
8	32	2	1	DST/ DS	16 +1/7	46, XX	N, XX
9	23	1	0	TST/ DS	17 +2/7	46, XY	N, XY
10	26	4	1	TST/ DS	16 +5/7	46, XY	N, XY
11	27	1	0	TST/ DS	16 +5/7	46, XX	N, XX
12	26	2	1	TST/ DS	19 +3/7	46, XY	N, XY
13	20	1	0	TST/ DS	19	46, XY	N, XY
14	26	1	0	TST/ DS	18 +5/7	46, XY	N, XY
15	33	5	2	TST/ DS	17 +3/7	46, XX	N, XX
16	28	2	1	TST/ DS	16 +6/7	46, XY	N, XY
17	40	2	1	AMA	18 +6/7	46, XX	N, XX
18	32	3	2	TST/ DS	18 +2/7	46, XX	N, XX
19	26	4	2	DST/ DS	16	46, XY	N, XY
20	18	2	1	TST/ DS	18	46, XY	N, XY
21	22	1	0	TST/ DS	18 +4/7	46, XX	N, XX
22	31	3	1	TST/ DS	17 +1/7	46, XY	N, XY
23	27	1	0	TST/ DS	17	46, XX	N, XX
24	25	3	2	TST/ DS	18 +3/7	46, XY	N, XY
25	28	3	1	DST/ DS	17 +2/7	46, XY	N, XY
26	43	6	2	AMA	19 +5/7	46, XY	N, XY
27	26	3	2	TST/ DS	19	46, XX	N, XX
28	27	3	1	TST/ DS	17 +4/7	46, XX	N, XX
29	32	2	1	TST/ DS	17	46, XY	N, XY
30	27	2	0	TST/ DS	17	46, XY	N, XY
31	20	1	0	TST/ DS	16 +5/7	46, XX	N, XX
32	44	4	1	AMA	18 +2/7	46, XX	Trisomy 21, XX
33	43	3	1	TST/ DS	17 +3/7	46, XY	Trisomy 21, XY
34	29	2	1	TST/ DS	16 +5/7	46, XY	Trisomy 21, XY
35	38	4	3	TST/ DS	17 +2/7	46, XX	Trisomy 21, XX
36	41	2	1	TST/ DS	18 +5/7	46, XY	Trisomy 21, XY
37	38	3	2	TST/ DS	18 +3/7	46, XX	Trisomy 21, XX
38	26	3	2	DS history of prior child	18	47, XY, +Y	N, XY
39	43	6	2	DST/ DS+AMA	16 +4/7	47, XY, +21	Trisomy 21, XY
40	25	1	0	TST/ DS	16 +2/7	46, XY	Hemorrhagic AF, No comment

N: Two signals each for chromosome 13, 18, 21, X, and Y; DS: Down's syndrome; AMA: advanced maternal age; TST: Triple Screening Test; DST: Dual Screening Test; AF: amnion fluid.

Although FISH and QF-PCR can be applied to all chromosomes, they are routinely used merely for 13, 18, 21, and sex chromosomes. Their rate of detection for all chromosome anomalies may be as low as 65% (3,11). The advantage of QF-PCR over FISH is that it can be used with less amniotic fluid and it is easier to perform. In addition, it can also detect maternal cell contamination. The FISH method is more effective than QF-PCR in mosaic subjects. When using the FISH method with non-hemorrhagic amniotic fluid, maternal contamination is not a serious problem because the geneticist can distinguish maternal contamination by investigating at least 100 cells.

The most important drawback of rapid tests is the problem of establishing mosaic cases and sex chromosome anomalies. In the present study a case identified as karyotype 47, XYY by conventional analysis was missed by QF-PCR.

QF-PCR and MLPA are more cost effective than FISH and can be used in larger series of patients. MLPA relies on the dose measurement of genomic sequences. Another advantage is that it can detect the 40 different genomic loci and mosaicism better than other tests (2).

As the sensitivity of rapid screening tests is as low as 70%-80% in mosaic cases and sex chromosomes, conventional cytogenetic analysis remains the method of choice for many patients. In the present study the sensitivity and specificity of QF-PCR was 83.7% and 50%, respectively. In addition, conventional karyotyping may detect numerical and structural anomalies other than trisomies (4).

In conclusion, given that a diagnostic test should be inexpensive, easily applicable, highly sensitive and specific, and reproducible when used in clinical practice, it can be stated that it is premature to use rapid tests in daily practice, because they can be used only for selected chromosomes (13, 18, 21 and sex chromosomes), have a high rate of failure, and maternal DNA contamination can influence the results.

For all rapid screening methods used during genetic counseling, it is important to inform patients that undergo aneuploidy screening by rapid screening methods about the advantages and disadvantages of the method in order to help them acknowledge the results in the event that an abnormality is identified in the karyotype. Rapid screening tests are never regarded as definitive.

Although the specificity and sensitivity values were not very high in the present study, in the future, following technological development of these tests, their efficacy may be expected to increase, shortening the waiting time before results are obtained and decreasing the anxiety experienced by waiting families. Nonetheless, until these advances are made, as these rapid techniques can detect only the numerical abnormalities of chromosomes 13, 18, 21, X, and Y, conventional karyotyping, which has a failure rate lower than 0.5% at present, will remain the gold standard for chromosome analysis.

References

1. Ogilvie CM, Donaghue C, Fox SP, Docherty Z, Mann K. Rapid prenatal diagnosis of aneuploidy using quantitative fluorescence-PCR (QF-PCR). J Histochem Cytochem 2005; 53: 285-8.
2. Hochstenbach R, Meijer J, van de Brug J, Vossebeld-Hoff I, Jansen R, van der Luijt RB et al. Rapid detection of chromosomal aneuploidies in uncultured amniocytes by multiplex ligation-dependent probe amplification (MLPA) Prenat Diagn 2005; 25: 1032-9.
3. Nicolini U, Lalatta F, Natacci F, Curcio C, Bui TH. The introduction of QF-PCR in prenatal diagnosis of fetal aneuploidies: time for reconsideration. Hum Reprod Update 2004; 10: 541-8.
4. Caine A, Maltby AE, Parkin CA, Waters JJ, Crolla JA. UK Association of Clinical Cytogeneticists (ACC). Prenatal detection of Down's syndrome by rapid aneuploidy testing for chromosomes 13, 18 and 21 by FISH or PCR without a full karyotype: a cytogenetic risk assessment. Lancet 2005; 366: 123-8.
5. Yılmaz Z, Özalp Ö, Tarım E, Üçkuyu A, Dağlı V, Şahin Fİ. Comparison of conventional cytogenetic and fluorescence in situ hybridization results of prenatal aneuploidy screening. Turk J Med Sci 2007; 37: 69-74.
6. Hulten MA, Dhanjal S, Pertl B. Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR. Reproduction 2003; 26: 279-97.

7. Evans MI, Henry GP, Miller WA, Bui TH, Snidjers RJ, Wapner RJ et al. International, collaborative assessment of 146000 prenatal karyotypes: expected limitations if only chromosome-specific probes and fluorescent in-situ hybridization are used. Hum Reprod 1999; 14: 1213-6.

8. Thein AT, Abdel-Fattah SA, Kyle PM, Soothill PW. An assessment of the use of interphase FISH with chromosome specific probes as an alternative to cytogenetics in prenatal diagnosis. Prenat Diagn 2000; 20: 275-80.

9. Cirigliano V, Voglino G, Canadas MP, Marongiu A, Ejarque M, Ordonez E et al. Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR. Assessment on 18000 consecutive clinical samples. Mol Hum Reprod 2004; 10: 839-46.

10. Mann K, Fox SP, Abbs SJ, Yau SC, Scriven PN, Docherty Z et al. Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. Lancet 2001; 358: 1057-61.

11. Evans MI, Ebrahim SA, Berry SM, Holzgreve W, Isada NB, Quintero RA et al. Fluorescent in situ hybridization utilization for high-risk prenatal diagnosis: a trade-off among speed, expense and inherent limitations of chromosome-specific probes. Am J Obstet Gynecol 1994; 171: 1055-7.