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Advantages and limitations of QF-PCR analysis in invasive prenatal genetic diagnosis: a tertiary center experience from Turkey

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Abstract

Objective: The aim of this study was to investigate the success and reliability of QF-PCR analysis in detecting chromosomal abnormalities and to determine its advantages and limitations.

Methods: Patients who underwent karyotype and QF-PCR analysis as a prenatal invasive diagnostic test in a tertiary center were retrospectively analyzed. Invasive genetic test indications, ultrasonographic fetal screening reports, karyotype and QF-PCR analysis results of the patients were obtained from the electronic data system. Karyotypes were classified as normal, common aneuploidies (trisomies 21, 18, 13, and sex chromosome aneuploidies) and other aneuploidies. QF-PCR analysis and karyotype results were compared for inconsistency.

Results: A total of 426 cases (41 [9.6%] chorionic villus sampling, 339 [79.6%] amniocentesis and 46 [10.8%] cordocentesis) were included in the study. The most common indication for prenatal invasive diagnostic testing was fetal structural anomalies (36.7%). Aneuploidy was detected in 61 (14.3%) of the fetuses. Fifty-nine (96.7%) of 61 fetuses with aneuploidy were common aneuploidies. The sensitivity and specificity of the QF-PCR analysis in detecting common aneuploidies was 100%. QF-PCR analysis was indicative if not diagnostic in all fetuses with mosaic trisomy or sex chromosome aneuploidies.

Conclusion: QF-PCR analysis is a rapid, robust, and reliable test for the prenatal detection of common aneuploidies. Although QF-PCR analysis has high sensitivity and specificity in detecting common aneuploidies, it should be used for rapid preliminary information and the result of karyotype analysis should be awaited for important clinical decisions.

Keywords: Aneuploidy, genetic counseling, karyotype, QF-PCR, rapid prenatal diagnosis.

Introduction

Numerical and structural chromosomal abnormalities are the most common causes of developmental disabilities and congenital malformations and are detected in approximately one in 200 newborns.[1] The current gold standard test for detecting numerical and major structural chromosomal abnormalities (>5 Mb) is fetal karyotype analysis.[2] On the other hand, one of the most important disadvantages of karyotyping is that cell culture is required for cytogenetic analysis of fetal samples and

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therefore results in a relatively long time such as 2 to 4 weeks.[3] As this long reporting time causes parental anxiety in this process, there has been an increased interest in developing molecular techniques for rapid prenatal diagnosis of common fetal aneuploidies to reduce reporting time and parental anxiety.

Trisomy 13, 18, 21, sex chromosome aneuploidies and triploidies account for more than 80% of major chromosomal abnormalities, and rapid detection or exclusion of these aneuploidies will shorten the anxious waiting period in most patients.[4] Three rapid aneuploidy tests are currently available for the detection of chromosomal abnormalities: fluorescent in situ hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), and multiplex ligation-dependent probe amplification (MLPA).[5–8] With these rapid aneuploidy tests, common aneuploidies (trisomies 21, 18, 13, and sex chromosome aneuploidies) can be detected in a period as little as 24 to 48 hours.

QF-PCR method relies on the amplification of polymorphic chromosome-specific DNA sequences (STR), and through fluorescent primers, amplified segments can be visualized and quantified as peak areas on automated DNA scanners.[9,10] This method has been proposed as a rapid, robust and reliable test for the detection of common aneuploidies and has been widely used in prenatal genetic diagnosis for over 20 years.[2–4,11–13] On the other hand, some authors underlined that QF-PCR kits are produced based on the Caucasian population and that STR markers may differ in any of the populations.[3,14] Considering this proposal, population-based studies are needed to evaluate the performance of QF-PCR in rapid prenatal diagnosis. However, there are limited studies evaluating the performance of QF-PCR in our country.[3,15,16]

The aim of this study was to investigate the success and reliability of QF-PCR in detecting chromosomal abnormalities in patients undergoing invasive genetic diagnostic tests and to determine the importance of QF-PCR in prenatal genetic diagnosis.

Methods

Patients who underwent prenatal invasive diagnostic tests (CVS, amniocentesis and cordocentesis) in a tertiary center between January 2021 and January 2022 were retrospectively analyzed. The study was approved by the ethics committee of our hospital. Patients for whom prenatal invasive diagnostic testing is recommended were given genetic counseling, including the limitations of the procedure, possible consequences, and complications, and informed consent was obtained prior to the procedure. Patients who underwent QF-PCR testing in addition to karyotype analysis were included in the study. Invasive genetic test indications, ultrasonographic fetal screening reports, karyotype analysis and QF-PCR results of the patients were obtained from the electronic data system of our hospital. Indications for invasive genetic test were classified as high risk in screening tests, advanced maternal age (>35 years), increased nuchal translucency (≥95th percentile of a reference range),[17] parents' anxiety, genetic abnormality in parents or previous child, fetal structural anomaly, suspected fetal infection, and the presence of soft marker in fetal ultrasonographic screening. Soft ultrasonographic markers were defined as nuchal fold thickness (≥6 mm), pyelectasis (≥4 mm), short femur and humerus (<2.5 percentile), hyperechogenic bowel, hyperechogenic cardiac focus, choroid plexus cyst, and hypoplastic or absent nasal bone.[18] Fetal ultrasonography was performed with the Voluson E6 (GE Healthcare, USA) device.

Fetal samples obtained by invasive procedure were divided into two parts. One part of the fetal samples underwent QF-PCR for rapid diagnosis, while standard karyotype was performed on the second part to validate QF-PCR. All fetal samples were compared with maternal peripheral blood samples to exclude maternal cell contamination.

In QF-PCR analysis, genomic DNA was extracted from fetal samples following the manufacturer's instructions. QF-PCR was performed for 13, 18, 21, X and Y chromosomes using the Aneusure kit (GeneTek Biopharma, Berlin, Germany) with 22 STR markers (AMXY, SRY, DXS7132, HPRT, DXS6803, DYS437, 7X, DXS981, D13S325, D13S252, D13S634, D13S258, D13S797, D18S390, D18S391, D18S1002, D18S535, D21S1809, D21S1446, D21FINAR, D21S1442, D21S1411). Fluorescently labeled PCR products were electrophoresed on the ABI-3130 genetic analyzer (Applied Biosystems, Waltham, MA, USA). The peak height ratio or area ratio was calculated for the QF-PCR results, and the peak area ratio between 0.8 and 1.4 between each allele was considered normal. The pres-

ence of two alleles of equal domain (1:1) was considered as normal disomy. Trisomic triallelic (1:1:1) and trisomic diallelic (2:1) results were considered abnormal. The presence of a single peak was considered non-informative and at least two compatible markers were required to obtain a result. Results were analyzed with Gene Mapper V4.0 (Softgenetics, State College, PA, USA).

In standard karyotype analysis, two or three cell cultures were performed on fetal samples taken from all cases and traditional G band was applied. Short and long-term cell cultures were used for the analysis of fetal samples. Routine evaluation included analysis of 20 random metaphase spreads from two independent cultures. Genetic analysis results were defined according to the International Human Cytogenetic Nomenclature System (ISCN 2020).[19] Karyotypes were classified as normal (normal karyotype, balanced translocation and de novo rearrangements without loss of genetic material), common aneuploidies (trisomies 21, 18, 13, and sex chromosome aneuploidies) and other aneuploidies (other trisomies, triploidy, deletions or duplications, de novo balanced rearrangements, unbalanced rearrangements, and mosaic trisomies). QF-PCR and karyotype results were compared for inconsistency.

The data analysis was performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA) package program. Descriptive data were expressed as number (%), mean ± standard deviation, as appropriate. Kolmogorov-Smirnov test was used to test the distribution of continuous data. The Student's t test was used to compare the values of two independent groups, since the distribution of the variables was normal between groups. The chi-square test was used to establish the statistical significance of categorical variables. Sensitivity, specificity, negative predictive values (NPV) and positive predictive values (PPV) were calculated for QF-PCR analysis. Statistical significance was determined as $p \leq 0.05$.

Results

A total of 535 prenatal invasive diagnostic tests were performed during the study period. Ninety-eight (18.3%) patients who did not undergo QF-PCR analysis, 4 (0.7%) patients with failed cell culture, and 7 (1.3%) patients with maternal cell contamination were excluded from the study. The remaining 426 patients (CVS in 41 [9.6%] patients, amniocentesis in 339 [79.6%] patients and cordocentesis in 46 [10.8%] patients) were included in the study. The flow chart of the patients included in the study is shown in **Fig. 1**. Aneuploidy was detected in 61 (14.3%) of the patients. The rate of aneuploidy was significantly higher in patients who underwent CVS compared to amniocentesis and cordocentesis (48.8% [n=20], 10.6% [n=36] and 10.9% [n=5] respectively, $p<0.001$). The mean gestational age was 12.95±0.84 weeks in patients who underwent CVS, 19.24±2.27 weeks in patients who underwent amniocentesis, and 24.87±2.55 weeks in patients who underwent cordocentesis. The mean age of the patients was 33.16± 6.11 years, and there was no significant difference between the mean age of patients with and without aneuploidy (33.65±1.16 and 33.13± 1.12 respectively, $p=0.751$).

Prenatal invasive diagnostic test indications and karyotype analysis results of the patients are shown in **Table 1**. The most common indication for prenatal invasive diagnostic test was fetal structural anomalies. The distribution of ultrasonographic findings of patients with fetal malformation in prenatal ultrasonography according to karyotype results is shown in **Table 2**. The most common structural anomalies in fetuses were congenital heart disease and central nervous system anomalies. Aneuploidy was detected in 69.6% of fetuses with hydrops fetalis and in 68% of fetuses with cystic hygroma, and these two were the anomalies most associated with aneuploidy.

Common aneuploidies were present in 59 (96.7%) of 61 fetuses with aneuploidy. The results of QF-PCR and cytogenetic analysis of common aneuploidies are shown in **Table 3**. When the analysis results were classified as normal and abnormal in common aneuploidies, the sensitivity and specificity of the QF-PCR analysis in detecting common aneuploidies were 100%. The genetic analysis results of the patients with inconsistency between the standard karyotype analysis and QF-PCR analysis are shown in **Table 4**. Although the results of the QF-PCR analysis were not reported as mosaic trisomy, the QF-PCR results were abnormal in all three mosaic trisomies. Similarly, the QF-PCR result was abnormal in a fetus with mosaic sex chromosome aneuploidy. Two (0.5%) fetuses with normal QF-PCR results had abnormal karyotype results other than common aneuploidies, and both fetuses had severe fetal malformations.

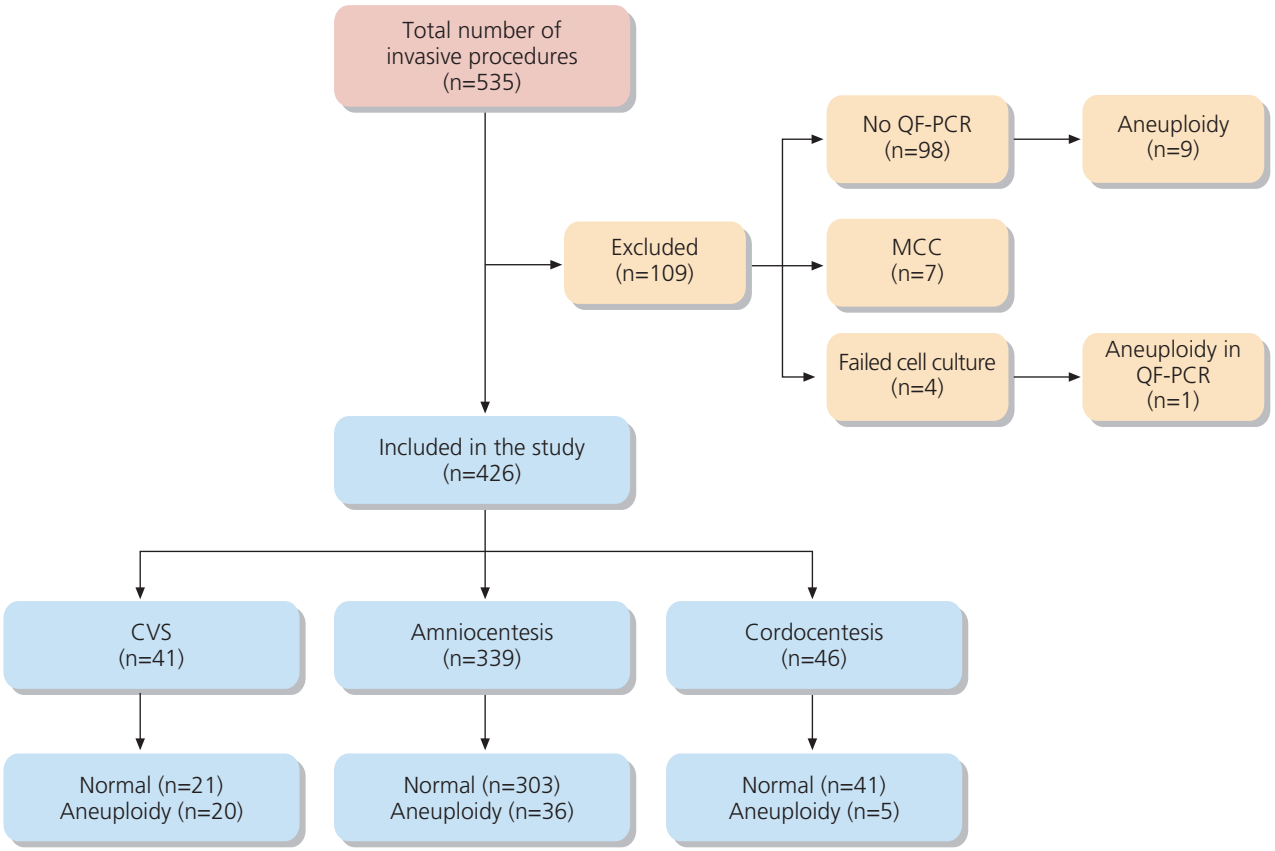

Fig. 1. Flow chart of the patients.

Table 1. Prenatal invasive diagnostic test indications and karyotype analysis results of the patients.

	Karyotype analysis results							
Indications	**Total (n=426) n (%)**	**Normal (n=365) n (%)**	**Trisomy 21 (n=32) n (%)**	**Trisomy 18 (n=16) n (%)**	**Trisomy 13 (n=4) n (%)**	**Sex chromosome aneuploidies (n=4) n (%)**	**Mosaic trisomies (n=3) n (%)**	**Other aneuploidies (n=2) n (%)**
Fetal structural malformation	160 (37.6)	109 (68.1)	24 (15.0)	16 (10.0)	4 (2.5)	2 (1.3)	3 (1.9)	2 (1.3)
High risk in screening tests	136 (31.9)	130 (95.6)	5 (3.7)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)
Presence of soft marker	55 (12.9)	53 (96.4)	2 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Advanced maternal age (≥35 years)	31 (7.3)	31 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Genetic abnormality in parents or previous child	16 (3.8)	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Increased nuchal translucency	13 (3.1)	11 (84.6)	1 (7.7)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)
Parents' anxiety	8 (1.9)	8 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Suspected fetal infection	7 (1.6)	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Values are presented as number and percentage (%).

Table 2. The distribution of ultrasonographic findings of patients with fetal malformation detected in prenatal ultrasonography according to karyotype results.

	Karyotype analysis results							
Ultrasonographic findings	Total (n=426) n (%)	Normal (n=365) n (%)	Trisomy 21 (n=32) n (%)	Trisomy 18 (n=16) n (%)	Trisomy 13 (n=4) n (%)	Sex chromosome aneuploidies (n=4) n (%)	Mosaic trisomies (n=3) n (%)	Other aneuploidies (n=2) n (%)
Congenital heart diseases	66 (15.5)	36 (54.5)	14 (21.2)	12 (18.2)	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)
Central nervous system anomalies	48 (11.3)	34 (70.8)	4 (8.3)	3 (6.3)	4 (8.3)	0 (0.0)	1 (2.1)	2 (4.2)
Skeletal system anomalies	42 (9.9)	27 (64.3)	3 (7.1)	8 (19.0)	2 (4.8)	1 (2.4)	1 (2.4)	0 (0.0)
Portal and umbilical cord anomalies*	36 (8.5)	24 (66.7)	3 (8.3)	6 (16.7)	1 (2.8)	0 (0.0)	1 (2.8)	1 (2.8)
Head and face anomalies	25 (5.9)	16 (64.0)	4 (16.0)	3 (12.0)	1 (4.0)	0 (0.0)	0 (0.0)	1 (4.0)
Cystic hygroma	25 (5.9)	8 (32.0)	7 (28.0)	7 (28.0)	1 (4.0)	1 (4.0)	1 (4.0)	0 (0.0)
Hydrops fetalis	23 (5.4)	7 (30.4)	6 (26.1)	7 (30.4)	0 (0.0)	2 (8.7)	1 (4.3)	0 (0.0)
Abdominal anterior wall defects	18 (4.2)	12 (66.7)	0 (0.0)	6 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Urinary system anomalies	16 (3.8)	12 (75.0)	2 (12.5)	0 (0.0)	1 (6.3)	1 (6.3)	0 (0.0)	0 (0.0)
Genital system anomalies	5 (1.2)	4 (80.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Congenital diaphragmatic hernia	5 (1.2)	3 (60.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal system anomalies	4 (0.9)	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Values are presented as number and percentage (%). *: Portal and umbilical cord anomalies include single umbilical artery, persistent right umbilical vein, umbilical cord cyst and ductus venosus agenesis.

Discussion

The present study provides a comprehensive evaluation of QF-PCR in rapid prenatal genetic diagnosis in the Turkish population. Considering that the main factor in the development of the QF-PCR was to achieve rapid and reliable prenatal diagnosis, our results show that QF-PCR provides rapid and reliable results in more than 99.9% of euploid and aneuploid fetuses. In the present study, there was complete consistency between QF-PCR and karyotype analysis results in 55 of 59 cases with common aneuploidy, and QF-PCR was indicative if not diagnostic in the remaining four cases. Furthermore, QF-PCR analysis did not yield false negative results in any of the cases. Therefore, we suggest that QF-PCR can be safely used in rapid prenatal genetic diagnosis in the Turkish population.

Table 3. Results of QF-PCR and cytogenetic analysis in common aneuploidies, and sensitivity, specificity, positive predictive value and negative predictive value of QF-PCR analysis.

				95% CI			
Fetal karyotypes	n (%)	QF-PCR	n (%)	Sensitivity	Specificity	PPV	NPV
Trisomy 21	32 (7.5)	Trisomy 21	32 (7.5)	100	100	100	100
Trisomy 18	16 (3.8)	Trisomy 18	18 (4.2)	100	99.5	88.8	100
Trisomy 13	4 (0.9)	Trisomy 13	4 (0.9)	100	100	100	100
Sex chromosome aneuploidies	4 (0.9)	Sex chromosome aneuploidies	5 (1.2)	100	99.7	80	100
Common aneuploidies	59 (13.8)	Common aneuploidies	59 (13.8)	100	100	100	100
All aneuploidies	61 (14.3)	All aneuploidies	59 (13.8)	96.7	100	100	99.5

Values are presented as number and percentage (%).

Table 4. Invasive genetic diagnostic test indications, fetal ultrasonographic findings and genetic analysis results of fetuses with inconsistency between standard karyotype analysis and QF-PCR analysis.

Cases	Indications	Ultrasonographic findings	Invasive procedures	Fetal karyotypes	QF-PCR
Case-1	Fetal structural anomaly	Subpulmonary perimembranous VSD Double outlet right ventricle Choroid plexus cyst	Amniocentesis	47,--,+18[48]/46,-- [2]	Trisomy 18
Case-2	Fetal structural anomaly	Bilateral pes equinovarus Hypoplastic nasal bone	Amniocentesis	47,--,+18[22]/46,-- [8]	Trisomy 18
Case-3	Fetal structural anomaly	Cystic hygroma Hydrops fetalis Agenesis of the ductus venosus	CVS	46,X,+18 [14]/45,X [36]	Monosomy X
Case-4	High risk in screening test	-	Amniocentesis	47,XXY [45]/46,XY [5]	XXY
Case-5	Fetal structural anomaly	Lissencephaly Agenesis of the corpus callosum Retrognathia Subaortic VSD Type B aortic interruption Thymus hypoplasia Agenesis of the ductus venosus Single umbilical artery Nuchal fold thickness Pelviectasis Hyperechogenic bowel	Amniocentesis	46,--, der(5)t(5;7)(p14.2;q22.11)	Normal
Case-6	Fetal structural anomaly	Lissencephaly Septum pellucidum agenesis	Cordocentesis	47,--,+der(22)t(11;22)(q23;q21)	Normal

CVS: chorionic villus sampling; **VSD:** ventricular septal defect.

There are conflicting results regarding the performance of QF-PCR analysis in prenatal diagnosis in the Turkish population. In a study with a relatively small number of cases, QF-PCR had poor performance in detecting common aneuploidies (Sensitivity: 50%, specificity: 83.7%, PPV: 14.3% and NPV: 96.9%), and the authors emphasized that conventional karyotype should remain the gold standard.[15] In a subsequent study involving 131 fetuses with aneuploidy, karyotype analysis and QF-PCR had similar success rates, and the authors suggested that QF-PCR could be preferred as the sole prenatal test in all indication groups without fetal ultrasonographic findings.[16]

The present study reveals that the performance of the QF-PCR analysis in the Turkish population is similar to the results of previously reported studies with a large number of cases.[2,13] In a study reporting the results of the nine-year experience of two centers in Spain and Italy that included a total of 43,000 prenatal diagnostic tests, QF-PCR showed 100% specificity for common aneuploidies, with PPV of 100% and NPV of 99.7%.[13] In another study involving 13,500 cases, QF-PCR was able to detect all 233 fetuses with common aneuploidy.[2] However, a small number of fetuses with aneuploidy were detected in these studies, which should be considered for predictive values. In our study, the rate of fetuses with aneuploidy was higher (14.3%) and we obtained similar predictive values.

In addition to providing fast and safe results, QF-PCR has advantages such as not requiring cultured cells and detecting maternal cell contamination.[11] In our study, the QF-PCR result of a fetus whose cell culture failed was reported as trisomy 21. In such a case, although QF-PCR has high sensitivity and specificity in detecting common aneuploidies, we suggest that the karyotype analysis result should be awaited in important clinical decisions such as termination of pregnancy. On the other hand, QF-PCR analysis can shorten the process of performing advanced molecular analysis in prenatal genetic diagnosis. Since microarray is a relatively expensive procedure, it is performed after karyotype analysis in fetuses with structural malformations

and waiting for the karyotype result prolongs the process. A strategy of performing microarray based on the QF-PCR result can avoid this delay. It has been suggested that as another advantage of QF-PCR, it can reduce the number of karyotype analyses and the cost of prenatal genetic diagnosis.[9,20] In a 2014 study conducted in southern Spain that included 928 cases, it was reported that a protocol consisting of a combination of QF-PCR and selective karyotype analysis reduced costs by 54% compared to karyotype analysis in all cases.[9] However, it is worth emphasizing that costs may vary, and we therefore think that each country should determine its own cost-effective policy. Despite all these advantages, there is still controversy about whether QF-PCR analysis can be used alone in prenatal genetic diagnosis.[2,9] Some authors suggested that QF-PCR analysis alone could be used to detect aneuploidies in selected populations.[8,13] QF-PCR analysis alone was introduced in London and South-East England region in 2007, and karyotype analysis was performed only in the presence of fetal structural anomalies, ≥2 soft markers for trisomy 21, nuchal thickness and familial chromosomal rearrangements.[21] This strategy reduced the need for karyotype analysis to 25%, with a detection rate of 99.9% for any chromosomal abnormality.[20] Since 2005, parents in Sweden can choose between QF-PCR alone or karyotype analysis when indications for prenatal testing are advanced maternal age, increased risk for a monogenic disorder or parental anxiety.[22] However, using QF-PCR alone in prenatal genetic diagnosis brings with it some ethical and medicolegal issues.

One of the major concerns for using QF-PCR alone is that the kits used in the analysis are designed to evaluate only chromosomes 13, 18, 21, X and Y. Therefore, QF-PCR analysis alone may not detect clinically significant abnormalities in other chromosomes. In previous studies, clinically significant non-trisomic chromosomal abnormality in the prenatal sample was estimated at approximately 1/1600 to 1/211.[2,20,23] Similarly, in our study, two cases (1/213) in which QF-PCR had normal results had other chromosomal abnormalities. However, we detected severe fetal structural malformations in both of these cases by ultrasonography. Therefore, even if the QF-PCR analysis was normal, karyotype analysis and microarray would be performed for both cases. Another concern for using QF-PCR alone is that it may not detect low levels of mosaicism (<20%) and has lower diagnostic performance in sex chromosome aneuploidies.[2,13,24] In our study, QF-PCR was indicative, although not diagnostic, in all fetuses with mosaic aneuploidy. In one case (Case-3), the QF-PCR result was reported as monosomy X, and the karyotype analysis result for this case was reported as mosaic trisomy 18 and monosomy X. Therefore, we suggest that an abnormal QF-PCR result must be confirmed by karyotype analysis, as there may be possible mosaicism. This is especially important for cases where placental sampling was performed by CVS. The clinician should consider that the abnormal result may be due to confined placental mosaicism, and the result should be confirmed by amniocentesis in the absence of ultrasonographic findings.[11]

In the present study, we evaluated prenatal chromosome analysis indications in our population and ultrasonographic findings in fetuses with aneuploidy. In previous studies, the most common indications were increased risk of aneuploidy on serum screening tests and advanced maternal age.[3,16] In contrast, the most common indication for chromosome analysis in our study was fetal structural malformations. This is probably related to the fact that noninvasive prenatal screening test (NIPT) is more widely used as a result of its decreasing cost. Our results support that parents prefer NIPT to avoid an invasive procedure in the absence of an ultrasonographic finding. On the other hand, considering that we detected aneuploidy in approximately 30% of fetuses with structural malformations in our study, we suggest that fetal ultrasonographic screening is one of the most important strategies for detecting fetal aneuploidies.

Our study had some limitations. First, although the number of cases included in the current study was relatively high, the number of cases with sex chromosome aneuploidy was small to evaluate the performance of QF-PCR in this group more accurately. Second, the current study could not assess whether a new protocol using QF-PCR analysis alone would be a cost-effective method. In a subsequent study, whether selective karyotype analysis to be performed in fetuses with ultrasonographic findings will be a less costly method and possible disadvantages of such a strategy can be investigated. On the other hand, the strengths of our study were that we conducted the study in a tertiary center

using standard protocols for prenatal genetic diagnosis and that prenatal ultrasonography was performed by experienced clinicians.

Conclusion

QF-PCR analysis is a rapid, robust, and reliable test for the prenatal detection of common aneuploidies. Although QF-PCR has high sensitivity and specificity in detecting common aneuploidies, we suggest that it should be used for rapid preliminary information and the result of karyotype analysis should be awaited for important clinical decisions. When the QF-PCR analysis is reported as normal, the residual risk of fetal aneuploidy is quite low. On the other hand, a normal QF-PCR result in fetuses with structural malformations must be confirmed by karyotype and microarray analysis. The clinician should interpret the results carefully and provide genetic counseling taking into account the limitations of QF-PCR analysis.

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