Original Article

Prenatal Screening for Aneuploidies Using QF-PCR and Karyotyping: A Comprehensive Study in Iranian Population

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Abstract

Background: We have investigated the efficacy of QF-PCR for the prenatal recognition of common aneuploidy and compared our findings with cytogenetic results in our laboratories.

Methods: A total of 4058 prenatal samples (4031 amniotic fluid and 27 chorionic villous samples) were analyzed by QF-PCR using several selected STR markers together with amelogenin. Results were compared to those obtained by conventional cytogenetic analysis.

Results: We detected 139 (3.42%) numerical abnormalities in our subjects by QF-PCR. Concordant QF-PCR and karyotype results were obtained in 4001 (98.59%) of the samples. An abnormal karyotype associated with adverse clinical outcome undetected by QF-PCR was found in 16.66% (n = 28) of samples. Using QF-PCR alone, we were able to detect abnormalities in 98.59% of all referred families; however the karyotyping results improved the detection rate to 99.85% of the referred cases. Individuals with neonatal screening result with 1:10 risk ratio showed 11.29% abnormal karyotype while this number was 2.16% in mothers with risk ratio of 1:250 or less.

Conclusion: In countries where large scale conventional cytogenetic is hampered by its high cost and lack of technical expertise, QF-PCR may be used as the first line of screening for detection of chromosomal abnormalities. We also recommend QF-PCR for all the families that are seeking prenatal diagnosis of single gene disorders aneuploidies screening to be added to their work up.

Keywords: Aneuploidy, cytogenetic analysis, Iran, QF-PCR, rapid prenatal diagnosis

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Introduction

In all countries, prenatal diagnosis (PND) of chromosomal aneuploidies is commonly performed by standard cytogenetic methods such as karyotyping. Due to chromosomal non-disjunction that results in the addition or deletion of a chromosome, the risk of most chromosomal abnormalities increases with maternal age. Chromosomal abnormalities occur in all cells and sometimes are also observed in mosaicism.[1] One of the advantages of karyotyping is to analyze structural chromosomal abnormalities such as balanced translocations and chromosomal inversion, which can be confirmed by high resolution banding techniques. However, the risk of this structural aberration doesn't correlate with maternal age.[2] Karyotyping has some limitations; embryonic cell cultivation is one of them, which can take around two weeks or even longer of culture of amniocytes *in vitro* to obtain enough cells in division for karyotyping and achieving the final result. It is recognized that long waiting times for results may cause much psychological suffering and this has been one of the main reasons for the introduction of molecular methods for prenatal diagnosis of common chromosome disorders.[2] In high-risk pregnancies when either parent is a carrier of a chromosome rearrangement such as a translocation, inversion or insertion, especially when the time of abortion is over and on the urgent need for appropriate information to be given to pregnant women (and their partners) regarding what fetal conditions may be looked for; and following discussions on the implications of the various disorders for child development, there is a need for a rapid and simple reliable prenatal diagnosis of targeted fetal chromosome disorders. Karyotyping errors are estimated to be about 4 to 14 in 1000 tested samples, 0.1 to 0.6 percent of this rate is due to contamination with maternal cells which lead to mistake in fetus sex determination. About 0.5% of this rate is due to the loss of fetal cells during cell cultivation and some percentages are related to laboratory errors.[3–7]

In recent years, fluorescent in situ hybridization (FISH) and quantitative fluorescence-PCR (QF-PCR) have been used for rapid PND (24 to 48 hours) of the most common aneuploidies in high-risk pregnancies.[8,9] FISH is a cytogenetic technique in which specific labeled DNA sequences are hybridized to complementary regions and lead to fluorescence emissions that can be observed by a fluorescent microscope. The major disadvantage of current FISH technology is the high cost. In addition, structural chromosomal abnormalities and mosaicism are not detected by this method. Since 1993, rapid diagnosis of common fetal aneuploidies of chromosome 13, 18, 21, X and Y has been evaluated through the examination of the short tandem repeat (STR) sequences in the genome using QF-PCR.[10]

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The sensitivity of QF-PCR for detecting chromosomal abnormalities is high. However, QF-PCR is unable to detect chromosomal structural abnormalities and mosaicism. To perform QF-PCR, a very small amount of amniotic fluid (AF) or chorionic villous sampling (CVS) is sufficient and there is no need for cell culturing. In the case of contamination of AF with maternal cells, it is possible to get accurate results by comparing maternal STRs with the fetal ones. A major problem with this method is that it is unable to detect chromosomal structural abnormalities and mosaicism.

Screening options in the first trimester include nuchal translucency (NT) testing in combination with measurement of pregnancy-associated plasma protein A (PAPP-A) and free β subunit of human chorionic gonadotropin (hCG). Screening options in the second trimester include serum screening using triple (free β-hCG, maternal serum alpha fetoprotein (AFP), unconjugated estradiol) or quadruple screening (hCG, inhibin A, maternal serum AFP, unconjugated estriol), and ultrasonography.[11]

Here we report the results of screening 4058 consecutive fetal samples, using both QF-PCR and conventional cytogenetic analysis. The detection rate in high-risk pregnancies determined by screening options in first and second trimesters of pregnancy.

Materials and Methods

A total of 4058 clinical specimens were referred to our center between October 2010 and October 2013. All of the women received genetic counseling, including detailed information on the advantages and limitations of the rapid QF-PCR assay. Routine informed consent was obtained in all cases. Ethical approval was sought from the institutional research board at Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran. The study adhered to the tenets of the Declaration of Helsinki. All participants signed an informed consent before being interviewed.

The clinical indications of prenatal diagnosis included: abnormal maternal serum screening (MSS) if the risk reached or exceeded 1 in 250, abnormal ultrasound (AU), AMA (≥ 35 years), family history of genetic/ chromosomal disorders (previous Down Syndrome (DS)), or prenatal anxiety. The majority of prenatal samples was AF (n = 4031), and collected between 12 and 28 weeks of gestation. The remaining samples were 27 CVS and collected between 11 and 13 weeks of gestation.

QF-PCR analysis

Genomic DNA was extracted from fetal cells, which were obtained from AF (1.5 mL) and CVS samples using the Chelex 100 kit (Insta Gene Matrix; Bio Rad; Cat, N732-6030). To compare the QF-PCR results with cytogenetic results, the rest of AF and CVS samples were used for cytogenetic analysis. The number of examined cells varied between 15 – 20. The results were reported within the first week.

QF-PCR was performed using different highly polymorphic STRs markers for chromosome 13, 18, 21, X and Y.[12,13] These highly polymorphic STRs were used to reduce the number of uninformative samples. A total of five STRs on chromosomes 13, (D13S631, D13S634, D13S797, D13S305, D13S258), five on 18 (D18S386, D18S390, D18S391, D18S535, D18S976), five for 21 (D21S1411, D21S1414, D21S1435, D21S1442, D21S1446), and one STR on chromosome X (HPRT), four for the pseudo autosomal regions PAR1 and PAR2 (AMXY, X22, DXYS218 and DXYS267), and one STR on Y chromosome (SRY) were selected.

Non-polymorphic sequence of the amelogenin gene (*AMXY*) and SRY were used which simultaneously allow the assessment of fetal sex. About 5 μL of the extracted DNA was applied in two multiplex PCR reactions using Aneufast QF-PCR kit (Aneufast Multiplex QF-PCR kit, Switzerland). PCR products were analyzed using the ABI3130 (Applied Biosystems, Foster City, CA) and Gene Mapper v.4 software was used to analyze of the fluorescent peak areas.[12] The criteria for the detection of a normal or pathological QF-PCR result was as follows: Peak area ratios between 0.8 and 1.4 were considered to be normal, whereas ratios above and below these were interpreted as trisomy; in addition, the presence of three alleles of equal peak area was interpreted as trisomy. In the case of detecting a trisomy, extra specific STR markers were analyzed to confirm the acquired result. Two peaks of equal length and area give normal result. The presence of a single peak was considered uninformative since two alleles were of the same length and the two peaks were superimposed. A single peak in all markers was considered to be monosomy. In cases where the AF had maternal contamination showing multiple triallelic peaks, precluding reliable diagnosis on fetal aneuploidy, DNA was extracted from mother's blood and analyzed alongside the AF sample. Fetal sex and chromosome X and Y copy numbers were determined in all cases by amplification of the non-polymorphic sequences of the amelogenin gene (*AMXY*) and SRY probe.

Cytogenetic analysis

Amniocytes were cultured and G banding was performed for all cases. Routine evaluation of each case involved the analysis of 20 random metaphase spreads from two independent cultures. Four metaphase spreads were photographed for karyotyping using the Leica imaging system. When mosaicism was suspected more than 50 metaphase spreads were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (2013) (ISCN2013).

Results

Validation specimens

The results of QF-PCR analysis of 4058 specimens are presented in Table 1 and compared with results obtained by conventional cytogenetic analysis. Comparing the QF-PCR results with karyotype analyses, there were no false positives and a single false negative. Due to laboratory error, the one specimen incorrectly classified as normal, was a Turner syndrome fetus. Also, the results of three-sex determination by QF-PCR were not compatible with karyotyping which was the result of a laboratory error. A total of 142 samples were contaminated with maternal cells. Maternal contamination could be overcome in 99 samples, while interpretation of QF-PCR results was impossible in 43 samples (just 1.05% of specimens).

Finally, 53 specimens could not be analyzed by QF-PCR giving a failure rate of 1.3%; maternal cell contamination (MCC) in 43 cases and low quality of extracted DNA in 10 cases (Table 1). We were not able to retest these samples because we received only a small aliquot of each and could not obtain any additional sample from the same individuals to repeat the experiment.

Patient demographics

A total of 4058 specimens (4031 AF and 27 CVS) were analyzed

Table 1. Comparison of QF-PCR and conventional cytogenetic analysis results in 4058 fetal samples.

Karyotype	Cytogenetic, N (%)	QF-PCR, N (%)
46,XX; 46,XY	3885 (95.73)	3862 (95.17)
47,XX +21; 47,XY +21	106 (2.61)	106 (2.61)
47,XX +18; 47,XY +18	12 (0.29)	12 (0.29)
47,XX +13; 47,XY +13	4 (0.10)	4 (0.10)
Turner syndrome (45,XO)	4 (0.10)	3 (0.07)
Klinefelter syndrome (47,XXY)	6 (0.15)	6 (0.15)
47,XXX	4 (0.10)	4 (0.10)
Triploidy (69,XXX; 69XXY)	4 (0.10)	4 (0.10)
Mosaics	11 (0.28)	0 (0)
Deletion	1 (0.03)	0 (0)
Insertion	1 (0.03)	0 (0)
Partial monosomy	2 (0.05)	0 (0)
Translocation	11 (0.28)	0 (0)
Laboratory errors	- - -	1 (0.02)[a] 3 (0.07)[b]
Unable to analysis	6 (0.15)	53 (1.30)
Total abnormalities	166 (4.09)	139 (3.42)
Test accuracy (%)	99.85	98.59

[a]45,XO; [b]Sex determination

Table 2. Number and percentage of aneuploidies detected in fetuses by different mother's age using QF-PCR.

Mother's age	Total samples, N (%)	Anomalies, N (%)	Normal, N (%)	Total aneuploidies detected, N (%)						
				47,**XX/XY** +21	47,**XX/XY** +13	47,**XX/XY** +18	45,**XO**	47,**XXY**	47,**XXX**	69,**XXX**, 69,**XXY**
< 35	2184 (53.82)	62 (2.84)	2122 (97.16)	40 (37.73)	4 (100)	7 (58.33)	3 (75)	4 (66.66)	1 (25)	3 (75)
≥ 35	1774 (43.72)	75 (4.23)	1699 (95.77)	65 (61.32)	0 (0)	4 (33.33)	0 (0)	2 (33.33)	3 (75)	1 (25)
Without maternal age	100 (2.46)	2 (2)	98 (98)	1 (0.94)	- - -	1 (8.33)	[a]	- - -	0 (0)	- - -
Total	4058 (100)	139 (3.42)	3919 (96.57)	106 (2.61)	4 (0.10)	12 (0.29)	3 (0.07)	6 (0.15)	4 (0.10)	4 (0.10)

[a]A Turner syndrome with false negative result by QF-PCR

prospectively by QF-PCR and karyopyping. The median maternal age was 33.3 years (range, 17 – 54 years) and 1774 (43.72%) women were 35 years of age or older (Table 2). The median gestational age was 18.3 weeks (range: 11 weeks to 25 weeks) (Table 3). The most common reason for referral was abnormal maternal serum biochemical screening results (3184, 78.46%), followed by advanced maternal age (1774, 43.72%) and abnormal ultrasound findings (220, 5.42%), (Tables 2, 3 and 4). A small proportion of patients were referred for other reasons such as having a previous child affected with a chromosome abnormality or another genetic disorder or being a carrier of a balanced chromosomal translocation (Table 5). It should be noted that some patients were referred for more than one indication and 654 individuals were examined directly without screening result (16.12%). In addition, some patients were referred for more than one indication; therefore, the percentages do not add up to 100%. Overall, 2974 (73.29%) fetuses had an estimated risk equal to or less than 1/250 (Table 4).

Among discrepant results between QF-PCR and cytogenetic analysis, those that could affect the clinical management of the pregnancy are shown in Table 1. In 11 cases QF-PCR failed to detect a translocation; 46,XY,t(1;11)(q33;q22.1), 46,XX,t(2;7)(q31.2;q31.2),46,XX,t(2;19)(q33.2;q13.31),46,XY,t(2;20)(q35;q13.1),46,XX,t(4;9)(p14;q21.2),46XY,t(5;7)(q31.1;q11.1),46,XX,t(5;20)(q21;q12),46,XY,t(8;16)(q24.12;q23.2), 46,XX,t(13;18)(q32;p11.2), 46,XX,t(14;17)(q11;q24), and 45,XX,der t(14;21)(q10;q10). In 11 cases QF-PCR failed to detect a mosaicism; 47XXY/46XY (in 3 cases with 20% – 36.36% trisomic cells), 45XO/46XY (monosomic cells: 33.33%), 47XY+mar/46XY (marker 29.16%), 47XX+mar/46XX (with 80% marker), 45XO/46XX (in 2 cases with 10% and 45% monosomic cells), 45XO/47XXX (with 40% trisomic cells and 60% monosomic cells), 47XY+9/46XY (with 44.44% trisomic-cells) and 48XXXY/46XY(tetrasomic cells: 61.54%). In addition cytogenetic detected one deletion (46,XX, del4p15.2), two partial monosomies (45,XY, der22t14; 22q13.2;p13, -14 a male fetus with partial monosomy of chromosome 14q11.1q13.2 and 46,XY, del2q32.3q33.2 a male fetus with partial monosomy of the long arm of chromosome 2q32.3q33.2) and an insertion (46,XY, ins8; 6q24.13;p24p21.3). In all cases that have a translocation, parental karyotype was analyzed and all discordant results, which indicated that the chromosomal abnormality was pathological, lead to the termination of pregnancy. Finally, 53 specimens (1.30%) had inconclusive results with multiple normal and multiple abnormal markers for at least one chromosome (Table 1). Therefore, a result was possible for 98.59% of all specimens received.

Table 3. Percentage of abnormalities in fetuses with different ages.

Fetus age (weeks)	Total No. tested	Detected No. (Abnormality)	Percentage of abnormality (%)
11	15	1 (+21)	ND[a]
12	12	1 (+21)	ND
13	10	1 (+21)	ND
14	47	7 (+21) 1 (+18)	17.02
15	190	16 (+21) 1 (+18) 1 (69,XXX) 1 (47,XXY) 1 (45,XO)	10.53
16	679	18 (+21) 3 (+18) 2 (69,XXX) 1 (+13) 1 (69,XXY) 1 (45,XO) 1 (47,XXX) 1 (47,XXY)	4.12
17	820	24 (+21) 2 (47,XXY) 1 (+18)	3.29
18	694	12 (+21) 3 (47,XXX) 1 (47,XXY) 1 (+18) 1 (45,XO)	2.59
19	438	9 (+21) 2 (+13) 1 (47,XXY)	2.74
20	359	10 (+21) 3 (+18)	3.62
21	253	2 (+21)	0.79
22	173	1 (+21)	0.58
23	138	1 (+18) 1 (+21)	1.45
24	118	1 (+13) 1 (+18) 1 (+21)	2.54
+25	112	2 (+21)[b]	1.78
Total	**4058**	**139**	**3.42**

[a]ND: Not detected, [b]One Turner case with false negative result.

Analysis of the heterozygosities of STR markers

The heterozygosities of selected markers for QF-PCR are given in Table 6. The markers D18S386, D21S1411, and D21S1442 showed a triallelic pattern, while the remaining markers showed a diallelic pattern. Among studied markers, D13S634, D18S976 and D21S1414 had the highest frequency of heterozygosity, therefore they were considered as the most informative markers. For sex chromosomes STR marker DXYS267 had the highest heterozygosity. We detected two cases with *AMXY* deletion, which is very low considering its frequency in the Iranian population. Moreover, we detected three cases with an additional Y chromosome (47,XYY), which are generally not clinically apparent. The incidence 47,XYY in our study was calculated 7.4 in 10000 in comparison with a general incidence of 10 in 10000. The minimum turnaround time was 24 hours and the maximum was 48 hours with an average of 36 hours. All aneuploidies detected by QF-PCR, was confirmed by cytogenetic results. We have also evaluated the correlation between the fetal age (weeks) and the number and type of the chromosomal abnormalities detected in our screening (Table 3). A total of 75 out of 1774 (53.95% of 139 detected abnormalities) identified fetuses with Down syndrome were found in women over 35 years of age, however this amount was 4/12 in trisomy 18 cases and 0/4 in trisomy 13 fetuses.

Table 4. Frequency of abnormalities for women with different risk ratio.

Risk ratio	Number of individuals	Risk for trisomies 13 and 18	Risk for trisomy 21	Results of QF-PCR			Total abnormalities, N (%)
				Detected abnormalities, N(Abnormality)	Normal fetus	Unable to analysis	
1/2-1/250	2974 (73.28)	124 (96.87)	2850 (93.25)	74 (+21) 6 (47,XXY) 5 (+18) 3 (69,XXX) 2 (47,XXX) 1 (+13) 1 (45,XO)	2851 (95.86)	31 (1.04)	92 (3.09)
1/251->1/400	210 (5.18)	4 (3.12)	206 (6.74)	0	208 (99.05)	2 (0.95)	0 (0)
Abnormality only detected in sonography	220 (5.42)	- - -	- - -	12 (+21) 3 (+18) 2 (+13) 2 (45,XO)	199 (90.45)	2[b] (0.91)	19 (8.64)
Without any screening result but advanced MA[a]	654 (16.12)	- - -	- - -	20 (+21) 4 (+18) 2 (47,XXX) 1 (69,XXY) 1 (+13)	604 (92.35)	20[c] 1[d] 1[b] (3.36)	28 (4.28)
Total	**4058 (100)**	**128 (100)**	**3056 (100)**	**139 (3.42)**	**3862 (95.17)**	**57 (1.40)**	**139 (3.42)**

[a]MA: Maternal age; [b]: Sex determination; [c]: Maternal cell contamination and low quality; [d]:(45,XO).

Table 5. Abnormalities detected by QF-PCR in mothers with different histories of abortions.

History of abortion	Total No.	Detected abnormal fetuses, N (anomaly)	Total aneuploidies, N (%)
Individuals with a history of 1 abortion	599	8 (+21) 2 (47,XXX) 1 (+18) 1 (+13) 1 (69,XXX)	13 (2.17)
Individuals with a history of 2 abortions	174	4 (+21) 1 (+18)	5 (2.87)
Individuals with a history of up to 3 abortions	56	1 (+21)	1 (1.78)
Total	**829**	**13 (+21) 2 (+18) 2 (47,XXX) 1 (+13) 1 (69,XXX)**	**19 (2.29)**

Discussion

Pregnant women with high risk of carrying infants with chromosomal anomalies due to maternal age, abnormal serum biochemical markers, or abnormal ultrasound findings require standard karyotyping of fetal cells obtained through amniocentesis or CVS culture. This method remains the gold standard prenatal diagnosis of chromosomal anomalies. The major disadvantage of this technique is the requirement for culturing the amniocytes, which takes 10 to 14 days for cells to grow and additional time for the analysis. In the last few years, QF-PCR has been applied for rapid detection of common aneuploidies and since then, it is becoming the method of choice for PND of chromosomal abnormalities. Our study presents the results of a large clinical application of QF-PCR for the rapid detection of autosomal and sex aneuploidies of chromosomes 13, 18, 21, X and Y on 4058 AF and CVS samples in Iranian families as an adjunctive test to conventional cytogenetic analysis. The majority of the pregnant women were referred to us because of positive screening test or increased risk of chromosomal abnormalities. The aim of this study was to identify women with an increased risk of carrying a fetus with certain congenital malformations or chromosomal abnormalities. The screening program can evaluate the risk of the fetus having trisomy 21, 18, 13 or an open neural tube defect. Women with pregnancies identified as positive when screening for these anomalies can then be offered a diagnostic test such as amniocentesis or CVS. The amniocentesis is performed at 15 weeks or later and takes 2 – 3 weeks to obtain a karyotype result. Counseling about the risks and benefits of invasive diagnosis is provided and the test is performed if the woman chooses this. Using QF-PCR alone, we were able to detect abnormalities in 98.59% of all referred families; however, the karyotyping results improved the detection rate to 99.85% of the

Table 6. Results of QF-PCR for each STR marker of chromosomes 13, 18, 21, X and Y.

STR	Chromosome Location	Mono allelic, N (%)	Diallelic, N (%)	Triallelic, N (%)
D13S258	13q21	847 (15.1)	4664 (83.20)	95 (1.70)
D13S305	13q12.1-13q14.1	1104 (20.65)	4203 (78.62)	39 (0.73)
D13S631	13q31-32	1338 (24.01)	4196 (75.32)	37 (0.67)
D13S634	13q14.3	823 (15.35)	4504 (84.01)	34 (0.64)
D13S797	13q32q33	1725 (36.16)	3025 (63.42)	20 (0.42)
D18S386	18q22.1	636 (11.50)	4478 (80.99)	415 (7.51)
D18S390	18q22.2	1942 (34.64)	3626 (64.69)	37 (0.67)
D18S391	18pter-1p11.22	1852 (32.88)	3747 (66.54)	33 (0.58)
D18S499	18q21.32-q21.31	2 (12.5)	10 (62.5)	4 (25)
D18S535	18q12.2	1283 (22.94)	4276 (76.47)	33 (0.59)
D18S858	18q21.1	4 (26.66)	6 (40)	5 (33.34)
D18S976	18q11.31	1092 (22.55)	3719 (76.75)	34 (0.70)
D18S1002	18q11.2	3 (20)	8 (53.33)	4 (26.67)
D21S1411	21q22.3	820 (14.61)	4551 (81.09)	241 (4.30)
D21S1414	21q21	959 (17.10)	4503 (80.30)	146 (2.60)
D21S1435	21q21	1268 (22.50)	4233 (75.14)	133 (2.36)
D21S1442	21q11.11	869 (17.91)	3789 (78.10)	194 (3.99)
D21S1446	21q22.3-ter	1258 (22.35)	4230 (75.18)	139 (2.47)
X22	Xp28 Yq (PAR2)	947 (17.33)	4443 (81.35)	72 (1.32)
DXYD218	Xp22.32 Yp11.3 (PAR1)	2005 (35.64)	3581 (63.66)	39 (0.70)
DXYS267	Xq21.31 Yp11.3 (PAR1)	1110 (23.05)	3668 (76.20)	36 (0.75)

referred cases. In this prospective study, we identified 166 cases of chromosomal abnormality with a frequency of 4.09% using both QF-PCR and karyotyping. The obtained frequency is higher than in another interventional study of 13,437 pregnant Iranian women with 51 detected chromosomal abnormalities (0.37%). Noteworthy, in this study three methods were used for screening including QF-PCR or multiplex ligation-dependent probe amplification (MLPA) and conventional karyotyping.[14] The results of recent study showed that 2824 (20.60%) women were 35 years of age or older (mothers of 2824 out of 13,706 fetuses); however, in our study there were 1774 women ≥ 35 years (43.72%). We could detect trisomy 21, 18, and 13 in 106, 12, and 4 fetuses, respectively, with frequencies of 63.85%, 7.22%, and 2.40%. In the study by Ghaffari, et al.[14] these frequencies were 33 (64.70%), 8 (15.68%), and 0% for three main abnormalities trisomy 21, 18, and 13, respectively. In our study, among 938 mothers (between 12 to 16 weeks of gestation) who were referred to our lab, we found 43 (4.58%) fetuses with Down syndrome and two (0.21%) fetuses with Klinefelter's syndrome. The prevalence of Turner syndrome (45,XO) is about 1 in 1500 at 12 weeks and 1 in 4000 at 40 weeks, which is compatible with our results. In this study, we detected 3 (0.07) fetuses with Turner syndrome (45,XO), and a false negative case, with a minimum fetal age of 15 weeks. For other sex chromosome abnormalities (47,XXX, 47,XXY and 47,XYY), there was no significant change with maternal age. In this study, we found 34 chromosomal aneuploidies among fetuses of 301 mothers with a high-risk ratio 1/10 (11.29%), which shows that the results of the screening methods were good. We had mothers with a risk ratio of up to 1/250 and among them, 92 fetuses with chromosomal aneuploidy was detected (3.09%).We did not detect any trisomy 21, 18, and 13 in 210 borderline women (risk ratio 1/250 to 1/400 (Table 4)). We found that the prevalence of Down syndrome in fetuses of mothers over 35 years of age was almost twice the prevalence of Down syndrome in fetuses of mothers less than 35 years, although the frequency of fetal trisomy in chromosomes 13 and 18 was higher in mothers less than 35 years of age.

Among our samples, 654 out of 4058 mothers had no screening results at all of whom we detected 4.28% of pregnancies with anomalies. These detected abnormalities were 20 Down syndrome, 4 Edward syndrome, 2 Triple X (47,XXX), and Turner syndrome (only detected by karyotyping), Patau syndrome, triploidy (69,XXY) each one case. About 220 of the individuals had sonography indications of which 8.64% had a fetus with aneuploidy (12 with Down syndrome, 3 Edward syndrome, 2 Turner syndrome, and 2 Patau syndrome). Abnormal concordant results were detected in 139 cases and cytogenetic analysis was capable of detecting an additional 10 cases.

At first, among our samples, 142 (3.49%) out of 4058 specimens had a sufficiently high level of maternal cell contamination (MCC) to preclude accurate interpretation of QF-PCR results. MCC of fetal material may arise during any of the invasive sampling procedure. Therefore, it is important to identify samples with MCC in order to avoid a diagnosis based on maternal cells. To avoid reporting of incorrect data, QF-PCR was performed for maternal blood samples of contaminated specimens and the results were compared with karyotype analyses. Finally, as a result of high MCC we could not come to a conclusion in 43 samples (43/142). Maternal cell contamination, if present, was always detected as characteristic QF-PCR patterns with extra peaks or skewed ratios for all chromosome markers that could not be confused with triploidy or mosaicism. In these cases, maternal blood samples and STR profiles of the fetal blood samples could provide valid and accurate information about potential maternal contamination so that a correct interpretation was possible.

In comparison to neighboring countries, markers X22 and DXYS218 showed highest frequency of heterozygosity in Turkey, and were considered to be the most informative markers.[15] In Egyptian population, SRY and AMXY markers gave successful diagnoses in all of the tested samples when compared to the cytogenetic results.[16] Moreover, for chromosomes 13, 18 and 21, D13S258, D18S386 and D211414-1411 had the highest heterozygosity in Turkish as well as Egyptian population, which was in agreement with our study, with the exception of D18S386 which showed a 7.5% triallelic pattern and indicated as uninformative for Iranian population (Table 6).[16] We detected two cases with *AMXY* deletion, which has been reported with a prevalence of 2 in 10000 in other populations and is considered to be the result of DNA polymorphism.[17] It is notable that, amongst the commonly recognized aneuploidy syndromes, such as trisomy 21, there is a high proportion of spontaneous pregnancy loss.[18] As Table 3 shows, this could be confirmed by comparing of the abnormality detection rate at 14 weeks (17.02%) with that at ≥ 25 weeks (1.78%).

In this study, 1.3% (53 out of 4058) of chromosome anomalies were not detected by QF-PCR. These included 11 mosaicism for aneuploidy of a chromosome not included in the QF-PCR kit and 11 translocation cases that would not be detected by the QF-PCR assay in addition to five other anomalies (deletions, insertion, partial monosomy, one false negative Turner syndrome) and three sex determination, because of MCC or low quality of the DNA sample.

Of these 53 cases, 11 (100% of translocation detected by karyotyping) were balanced translocations, inherited from a phenotypically normal carrier parent, which were *de novo* in the fetus. In eleven individuals, cytogenetic analysis revealed sex chromosome mosaicism, whereas the QF-PCR failed to detect this, even in cases with more than 20% trisomic cells. However, some other studies reported that the cell lines contributing at least 20% of the total cell population can be confidently identified by QF-PCR.[19] In order to provide a rapid and accurate result abnormalities detected by QF-PCR were double checked with the FISH method, and the families were provided with genetic counseling. Therefore, the family did not have to wait for completion of cytogenetic analyses.

In this study, we report the successful use of QF-PCR with CVS specimens (27out of 27), and thus, it is expected that the technology could easily be validated for CVS.[20,21]

To date, this is the largest study in Iran that has prospectively evaluated the performance of QF-PCR in the prenatal diagnosis of aneuploidy for chromosomes 13, 18, 21, X, and Y. Several large studies have now been published that indicate that QF-PCR is a sensitive and specific method for detecting aneuploidy in prenatal samples.[22–24] These studies suggest that QF-PCR shows a very high level of concordance with conventional cytogenetic studies (99.6% in the largest study to date) with no false positives and very few false negatives.[21,25,26] Our QF-PCR results are consistent with these large studies and indicate 98.6% concordance with conventional cytogenetic studies, no false positives and only one false negative result. Considering abnormal results, QF-PCR detected 83.7% of all anomalies in our study, which is comparable with other studies (92.3%). In addition, we could detect 99.3% of all clinically significant anomalies, which is comparable with the rate reported in another large study (95%).[21] The amplification failure rate for other reported studies ranged between 0.05% and 0.09%, which is comparable with our results (1.31%).[21,25] Interpretation of QF-PCR results was impossible due to extensive MCC in 1.05% of our cases. This amount reduced to 0.28% when cases were re-analyzed and confirmed by karyotyping results. However, some other studies have reported extensive MCC rate in 1% – 1.7% of specimens.[21,26]

Recently, Kong, et al.[27] developed a segmental duplication quantitative fluorescent polymerase chain reaction (SD-QF-PCR), for the prenatal diagnosis of fetal chromosomal aneuploidies. This method is based on the co-amplification of segmental duplications located on two different chromosomes using a single pair of fluorescent primers. The results were entirely consistent with the previous results of conventional cytogenetics, therefore this procedure represents a competitive alternative diagnostic tool for use in prenatal screening.[27]

In conclusion, the present results confirm that QF-PCR is a rapid, simple and accurate prenatal diagnostic test. The application of this test is ideal in countries with high rates of consanguinity.[28] We can also conclude the national natal screening program is extremely efficient and being accurately conducted in the nation. In addition, karyotyping is also recommended in all prenatal cases to increase the power of detection of aneuploidies.

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