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Prenatal diagnosis of fetal aneuploidies usin g QF-PCR in 333 cases

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ABSTRACT

Introduction: The most common chromosomal abnormalities detected in perinatal period are aneuploidies of chromosome 21, 18, 13, X and Y. The aim of this study is to assess referral reasons for invasive diagnostic method using rapid QF-PCR for fetal chromosomal abnormalities in gynecologists' referrals.

Methods: A retrospective study of results was performed on data between September 2015 and July 2016 at Mashhad Hope Generation Genetic Polyclinic for genetic examination. In order to prenatal diagnostic procedures, 333 amniotic fluid or CVS samples were received in our genetics polyclinic. DNA were analyzed with the QF-PCR technique by employing 26 short tandem repeat (STR) markers to detect chromosomes 13, 18, 21, X and Y aneuploidies.

Results: In 9 (2.7%) samples only one aneuploidy was detected. The aneuploidies included trisomy 13 (N= 1, 0.3%), trisomy 18 (N= 1, 0.3%), trisomy 21 (N= 6, 1.8%), monosomy X (N= 1, 0.3%). Referral reasons for positive results showed abnormal 1st and 2nd trimester screening (2.1%), history of previous pregnancy with chromosomal anomalies (0.3%) and hypoplastic NB (0.3%). Since P-value was considered as <0.05, relation between QF-PCR results and referral reasons were significant**.**

Conclusion: In conclusion, prenatal diagnosis is crucial for management of a high risk pregnancy. QF-PCR is reliable, accurate, and robust, but it has a limitation of not to able to examine full genome. This method is the fastest diagnostic test for prenatal diagnosis especially in the context of a high risk pregnancy and so it provides less stressful period for pregnant women.

Key words: QF-PCR, Prenatal diagnosis, Aneuploidy

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Introduction:

Prenatal screening and diagnosis is important in the new era of diagnosis and management of genetic disorders in obstetrics (1). Chromosomal aneuploidies cause a number of syndromes which are generally associated with severe mental retardation, dysmorphic features, growth and developmental delay, etc (2). The prenatal diagnosis of chromosome aneuploidies is typically performed using cytogenetic analysis of cultured amniocytes, chorionic villus or fetal blood cells. Although this conventional cytogenetic technique is gold standard for the detection of fetal chromosomal abnormalities but is time consuming (up to 2 weeks) (3). A rapid diagnostic method is vital in the case of an abnormal ultrasound or biochemical findings, which mainly suggests one of the most common aneuploidies for chromosomes 21, 18, 13, or X (4). It has been proven that quantitative fluorescence polymerase chain reaction (QF-PCR) is a cost-effective, robust and accurate rapid prenatal test for common aneuploidies with a reporting time about 2-3 days. QF-PCR enables the determination of parental origin of chromosomes as well (5). This method uses short tandem repeats (STR) for the detection of aneuploidy and it has been validated and successfully applied by a number of UK and European labs. The rapid result may be followed by a confirmatory test such as full karyotype analysis on cultured cells (6). A number of studies on large clinical data sets have documented the diagnostic potency of QF-PCR for prenatal diagnosis of common aneuploidies using amniotic fluid (AF) samples. In addition, many countries apply QF-PCR as a stand-alone test (7). The aim of our study is to assess the referral causes in pregnant women in different gestational weeks and QF-PCR results.

Methods:

Participants and data collection

The selected referrals were 333 pregnant women from September 2015 until July 2016, with an increased risk for fetal aneuploidy based on following: the first/ second trimester abnormal screening, Increased nuchal translucency (NT), Increased nuchal fold (NF), hypoplastic nasal bone (NB), maternal age, history of previous abnormal pregnancy and intra uterine growth retardation (IUGR). All samples were analyzed at the Mashhad Hope Generation Genetic Polyclinic for genetic examination. Clinical information and pregnancy status were collected from the genetic test request form.

DNA extraction and genetic analysis

Fetal DNA extracted using QIAamp DNA Mini Kit from amniotic fluid and chorionic villus samples. QF-PCR applied to 26 short tandem repeat markers on the chromosomes 13, 18, 21 and genes X and Y chromosomes. Using Devyser compact V3 QF-PCR kit, samples were tested for aneuploidies of the chromosomes 13, 18, 21, X and Y. PCR reaction was performed as follows: in a total reaction volume of 25 µL containing 20µL 1×Reaction Master Mix and 5 ng genomic DNA. The reaction mixture was preheated at 95°C for 15 minutes, subsequently 27 cycles of 30 seconds at 95°C, 90 seconds at 60°C, and 90 seconds at 72°C with a final extension step at 72°C for 30 minutes. All electropherogram peaks were evaluated on ABI3130. Data analysed using Gene marker v2.6.3. Data were analyzed using SPSS 20.

Results:

An overview of all samples according to referral classification is shown in figure 1. As shown in Figure.2, maternal age mean was 32.64 years (16-45 yrs). Gestation week mean was 18W4d (14-28 weeks). Among 333 samples received, in 9 (2.7%) samples an aneuploidy was detected. The aneuploidies included trisomy 13 (N = 1, 0.3%), trisomy 18 (N= 1, 0.3%), trisomy 21 (N = 6, 1.8%), monosomy X (N= 1, 0.3%) (figure 3). As summarized in Table 1, referral reasons for positive results showed abnormal 1st and 2nd trimester screening (2.1%), history of previous pregnancy with chromosomal anomalies (0.3%) and hypoplastic NB (0.3%). Since P-value was considered as <0.05, relation between QF-PCR results and referral reasons were significant.

Table 1. Statistical data of pregnant women according to referral reason classification

Classification	All pregnancies (N=333)	
	N	%
Maternal age at sampling (yrs)	32.64 (16-45)	9.3
Abnormal 1st & 2nd maternal serum screening	179	55.9
History	7	2.4
Hypoplastic NB	20	6.3
increased NF	6	1.8
increased NT	4	1.2
IUGR	2	0.6
an abnormality	30	9
Multiple abnormalities	45	13.5

NB = nasal bone, NT= nuchal translucency, IUGR= intra uterine growth retardation, NF = nuchal fold

Table 2. Relation between QF-PCR results and referral reasons

	Abnormal 1st & 2nd maternal serum screening		History		Hypoplastic NB	
	N	%	N	%	N	%
Trisomy 13	1	0.3				
Trisomy 18					1	0.3
Trisomy 21	5	1.5	1	0.3		
Monosomy X	1	0.3				
P-value	<0.05		<0.05		<0.05	

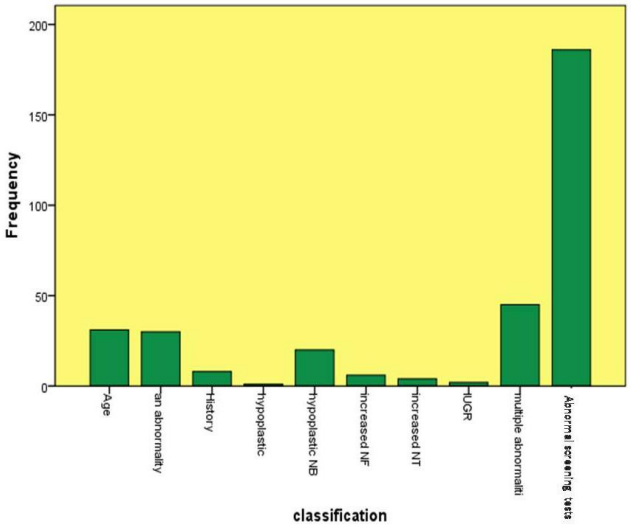

Figure 1. Classification of referral reasons for QF-PCR testing

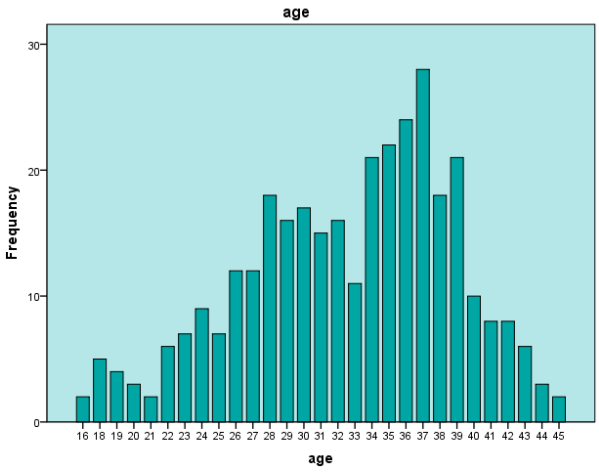

Figure2. Frequency of referral reasons according to maternal age

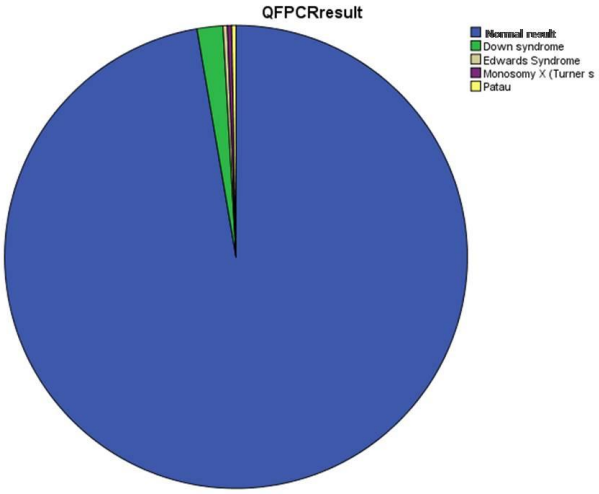

Figure 3. Distribution and types of aneuploidy detected in the cohort of 333 referral samples

Conclusion:

QF-PCR has been applied in rapid aneuploidy diagnosis for almost twenty years. This technique has been improved and widely used nowadays. It has many advantages, such as accurate, easy to manipulate, quick to generate results, high throughput to test samples, cost effective, etc (8,9). A number of studies have reported that, QF-PCR can be tested on different fetal samples such as CVS, AF, fetal blood, and fetal tissue obtained after termination of pregnancy for detecting all common aneuploidies (10,11). In our retrospective study, high risk pregnant women for chromosomal anomalies due to maternal age, abnormal 1st and 2nd trimester serum screening, one or multiple abnormalities, Increased NT/NF, hypoplastic NB, history of previous abnormal pregnancy and IUGR, were assessed for QF-PCR analysis. The quantity of DNA obtained from fetal cells is usually adequate and clean therefore suitable for testing by QF-PCR. Our results showed common aneuploidies in 2.9% of the samples. All aneuploidy were detected during 18w to 19w of pregnancy which showed the most detected aneuploidies were diagnosed in second trimester as reported in previous studies (12). The referral reason according to advanced maternal age (35-45 yrs) was 45% which is compatible with the other reports (13).

In conclusion, prenatal diagnosis is crucial for management of a high risk pregnancy. In this regard, we focused on chromosomal aneuploidies and how common these abnormalities occur during pregnancy. Although karyotyping of fetal cells obtained through amniocentesis or CVS culture remains the gold standard prenatal diagnosis of chromosomal anomalies, the main disadvantage of this method is need for culturing the amniocytes and needs for about two weeks for cells growth and additional time for the analysis (14). QF-PCR is a reliable, accurate, and robust, but it has a limitation that it maynot be able examine full genome. This method is the fastest diagnostic test for prenatal diagnosis especially in the context of a high risk pregnancy and so it provides less stressful period for pregnant women.

Conflict of interest

The authors declare that they have no competing interests.

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