A FALSE NEGATIVE QF-PCR AND TRISOMY 18-TRISOMY 9 MOSAICISM

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SUMMARY

Using QF-PCR for rapid prenatal diagnosis of major chromosome aneuploidies demonstrated that the method is highly efficient, reliable and cost effective in the literature. A discrepancy has been showed between QF-PCR and karyotyping results as 0.2% in mosaicism.Our case was in 17 th gestational week who referred to our clinic for having a trisomy 18 risk as 1 in 50 in triple test.On the ultrasonography, we detected bilateral choroid plexus cysts and bilateral pyelectasia.After amniocentesis QF-PCR has been showed a normal chromosomal patern and cytogenetic analysis has been showed trisomy 18 and trisomy 9 mosaicism.

Key words: *mosaic trisomies, prenatal diagnosis, QF-PCR method*

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ÖZET

YANLIŞ NEGATİF QF-PCR VE TRİZOMİ 18-TRİZOMİ 9 MOZAİZM

Major kromozomal anöploidilerin hızlı prenatal tanısında Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) yöntemi literatürde oldukça etkin (%95), güvenli ve düşük maliyetli olarak gösterilmiştir. Mozaik trizomilerde QF-PCR ve karyotipleme yöntemleri arasında % 0.2 oranında uyumsuzluk varlığı bildirilmiştir. Bizim olgumuz; 17. gebelik haftasında triple testinde trizomi 18 riskinin 1/50 olması nedeniyle kliniğimize gönderilmişti. Yapılan ultrasonografide fetusta bilateral koroid pleksus kistleri ve bilateral pelviektazi olduğu saptandı. Amniotik sıvıdan yapılan QF-PCR analizi; normal kromozomal yapı gösterdi ancak sitogenetik analizde fetusta trizomi 18 ve trizomi 9 mozaizm olduğu tespit edildi.

Anahtar kelimeler: *mozaik trizomiler, prenatal tanı, QF-PCR yöntemi*

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INTRODUCTION

Prenatal diagnosis of chromosomal abnormalities is performed by cytogenetic analysis during the last four decades, and during the last two decades they are diagnosed by Fluorescence In Situ Hybridization (FISH) analysis. For the cytogenetic analysis appropriate banding and painting operations following in-vitro culture that lasts for days is performed during the metaphase stage. Chromosome analysis performed with this analysis provides precise determination of all the numerical and structural abnormalities of the chromosomes(1). The main disadvantage is that the culture of fetal cells takes a long time and the time elapses from taking the sample

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to receiving the result (an average of 14 days) might cause anxiety in the family. This situation was shown to be more pronounced in patients that have an increased risk of chromosomal disorders according to non-invasive screening tests (biochemical and / or ultrasound) during the first or second trimester[2].

Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) analysis determines the major numerical abnormalities (chromosomes 13,18,21, X and Y) within a few hours after the sample is taken, which is a method that had been used in the past 15 years[3]. The analysis of the trisomies in the amniotic fluid is based on reproduction of the chromosome specific small DNA sequences (STR markers containing 3,4,5 repeats) and capillary electrophoresis analysis. While using selected chromosome-specific 17 STR markers they are diagnosed according to 1.2 or 3 peaks they create and with the analysis of gap between size and space with a special program[1,4-6]. This method, which was first used by Mansfield in 1993 in the analysis of Down syndrome and other aneuploids, is being used in the routine check after analyzing thousands of different populations. In the Turkish population in 2007 Onay et al compared both the QF-PCR and cytogenetic results of the amniotic fluid and found similar results to other populations[5]. In a study presented by Ozkinay et al in 2008 the results of a 3-year audit was shown to be compatible with the literature[7]. However, mosaicisms that have a low rate (less than 10%) might not be recognized[8,9]. In this study, we presented a case that could not be diagnosed with QF-PCR following amniocentesis but trisomy 18 and trisomy 9 mosaicism detected with karyotyping.

CASE REPORT

23-year-old17-week pregnant woman gravida 2, parity 1 was referred to our clinic for amniocentesis because the risk of trisomy 18 in the triple test was reported as 1/50. In the triple test, serum alpha-fetoprotein was 27.96 IU / ml (0.81 MOM), uE3 1 ng / ml (0.80 MOM) and hCG 18,213 mIU / ml (0.46 MOM) and the risk of trisomy 18 was calculated as 1/50. The detailed obstetric ultrasound revealed bilateral pyelectasis and bilateral choroid plexus cysts in the fetus. The choroid plexus cyst was measures as 6 mm in the right and 5 mm in the left. The presence of pyelectasis was determined 7 mm in the right and 5.9 mm in the left by the measurement of anterior-posterior diameter of the pelvis in the transverse cross-section of abdomen. Because of the patient's anxiety due to the possibility of trisomy and after she accepted the risk of abortion due to amniocentesis, amniocentesis was performed. 48 hours following the amniocentesis QF-PCR result performed by patented Gene Scan program using ABI 3100 revealed a normal chromosomal pattern (with 4 informative STR marker) (Figure 1). Because of the existence of two different ultrasonographic findings and high risk in the triple test result cytogenetic analysis was waited in order to reach a definite decision. On the 15th day of amniocentesis mosaic structure of Trisomy 18+Trisomy 9 was detected (Figure 2,3). Proportion of abnormal cells in the karyotype was 10% in total and the structure of 86% of these trisomic cells was 47XY +18, and 14% of them were 47 XY+9[10]. Amniotic cells were inspected in 2 separate flasks. In the first flask 50 cells were inspected; 44 of them were 47, XY +18 and 6 of them were 47, XY +9. In the second flask 20 cells were inspected; 16 of the were found as 47, XY +18 and 4 of them were 47, XX+9. This situation was thought to be compatible with 3rd grade mosaicism. The patient was told that cordocentesis should be performed for a definitive diagnosis, but she did not accept this process. The patient was informed about the prognosis of mosaicism, however, the pregnancy continued since she did not accept termination of the pregnancy.

In the ultrasonography performed during the 21st week of pregnancy, an expansion in the posterior fossa of the fetus was observed (foramen magnum was measured as 12 mm). Bilateral choroid plexus cysts and pyelectasis were persisting in the same sizes. During the follow-up pregnancy continued without problems until term. The patient had a vaginal delivery in a special center and the baby was ex postpartum at the 4th hour.

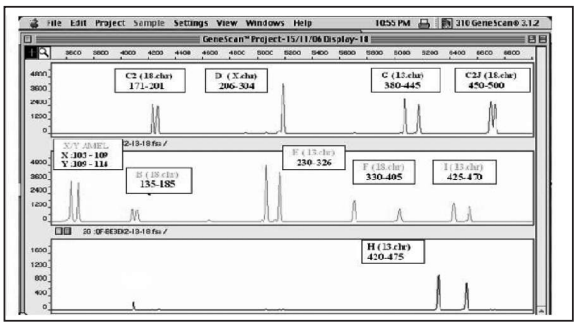

Figure 1: *QF-PCR analysis of chromosones 13,18 and the sex chromosomes.*

**There is no maternal contamination*

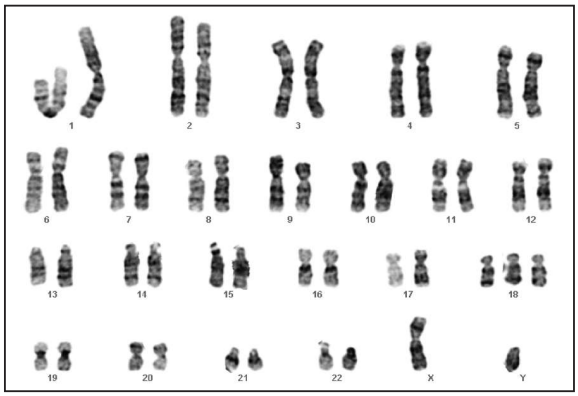

Figure 2: *Metaphase plates, trisomy of 18th chromosome is shown with G banding.*

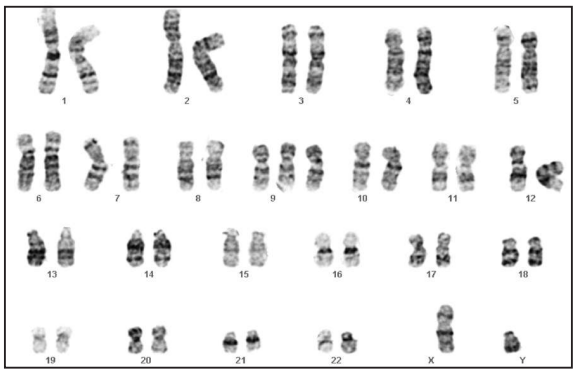

Figure 3: *Trisomy 18 is seen in karyotyping.*

DISCUSSION

In the early 1990s fluorescence in situ hybridization (FISH) and very recently QF-PCR are the two methods used for prenatal diagnosis in order to address the need of fetal cell culture. They provide rapid diagnosis of some specific chromosomal abnormalities[1-10]. FISH is being performed by using specific labeled DNA probes that are detectable using chemically modified and directly emitting fluorescence or could be fixed by a connecting molecule emitting fluorescent[5-11]. Normally, when the nuclei of fetal cells are analyzed with fluorescent microscopy analysis two points would be observed for each one of the chromosomes that is examined. Trisomies have an extra point, and monosomies lack one point[6-12].

The most commonly used form of QF-PCR is chromosome-specific, containing replications of repeats of small 3.4, or 5 nucleotide DNA in the repetitive DNA sequences. Repeated short DNA sequences are standardized and polymorphic, and the length of them varies significantly among the people like a fingerprint according to repeated triple, quadruple or quintet nucleotides. DNA is replicated by using fluorescent primers of PCR and this product could be seen with a gene browser program as each length repeated using automated DNA sequencer might be seen as the peak area and are comparable[8,9]. 65-95% of the DNA replicated from the normal people is heterozygote (includes different lengths of alleles) and are expected to have two peaks in the same area[7]. DNA replicated from the trisomic people;might reveal and extra peak in the same regions in the triallelics and only two peaks in the diallelics and one might be twice as long as the other[7]. Because QF-PCR is a method that works with duplication of DNA and isolated DNA was amplified at least for 20 cycles it fails show a difference in the DNA below 20%[1-5]. This method can also be applied to different fetal tissues (amniotic fluid, fetal blood, chorionic villi and fetal tissues after termination) and might show us the maternal contamination with blood samples taken from the mother. result can be given by working with at least 2 informative markers for each of the chromosome number[13-18]. In our case, DNA markers were informative and the patient had intermarriage.

FISH and QF-PCR quickly show whether the examined chromosomes are aneuploidy or not (24-48 hours). Both methods might be used in the recognition of all the chromosomes, but they were developed for the routine use of 13, 18, 21 chromosomes and sex chromosomes. These methods could be used for the high-risk patients (fetal malformation / soft marker are determined), or for the patients in the advanced weeks of pregnancy that are late for cytogenetic analysis. They are reliable to intervene when a chromosomal abnormality is detected, but some geneticists might accept them as a start while waiting for the result of karyotyping. In fact, the chromosome disorders studied with these rapid survey techniques consist only 65% of changes observed in high-risk population[14]. QF-PCR method contains some advantages compared to the FISH method. QF-PCR analysis provides examinateion of a much larger number of cells compared to FISH. Since this process could be easily automated numerous samples could be studied at the same time and the whole process takes only 30 minutes. In the FISH method there are specific probes and they do not require any authentication[9-14]. While QF-PCR might define maternal cell contamination, FISH might not revealthis for the female fetuses. For these reasons, QF-PCR is being rapidly accepted for the prenatal diagnosis as an alternative to conventional

cytogenetic analysis[10-16]. Like some other prenatal diagnostic tests QF-PCR cannot define the mosaicism with a low rate. Low mosaicism rates (less than 10%) could not be defined by molecular testing[4-8]. The ability of QF-PCR in sensitivity of defining two or more cell lines the mosaic patients depends on the rate of mosaic cells and the involved chromosomes. For example, 46, XX/45,X mosaics could be diagnosed with an unbalanced ratio using X chromosome markers, when at least 20% of cells of aneuploids are seen. In some mosaic fetuses containing 46, XX/45,X or 45,X/47XXX or 46, XX/45, X/47, XXX, determination the type and rate of different cell groups in fetuses is quite difficult. Because they can create fluorescent peaks like the normal 46, XX or 47, XXX[17,18]. In some cases, the results of QF-PCR and cytogenetic analysis in X chromosome mosaicism discrepancies might occur depending on the ratio of abnormal small cell population. This discrepancy rate has been reported as 0.2% in the literature[1]. This is because of the differences in the in vitro growth rate of normal (46, XX or 46, XY) and 45,X cells. Aneuploid cells grow faster than the normal cells. However, in the presence of more than one cell line QF-PCR allows diagnosis in the 50% of the mosaic trisomies that were diagnosed with cytogenetic analysis[1].

In conclusion, in the literature when QF-PCR method is used alone as the standard method it might miss the single chromosome abnormalities that have an increased frequency with the advanced age according to distribution of age. The rate of this is 1 in every 150 abnormal karyotype and one in every 10-30000[13-18]. Even though, it might miss some sex chromosome abnormalities, mostly structural, in the literature it has been emphasized that this error rate is acceptable[8-13].

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