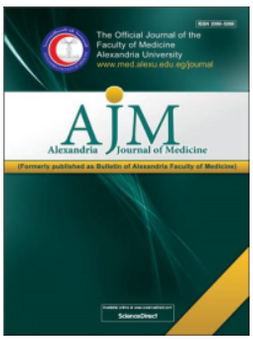

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Confirmatory test versus screening test analyses for fetal mosaic variations; a large scale study

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ABSTRACT

Background: Mosaic genetic anomaly is a problematic and interpretative issue in prenatal diagnosis. Conventional karyotyping, as a confirmatory test traditionally used for detecting mosaic and nonmosiac prenatal disorders. Recently Quantitative Fluorescence PCR (QF-PCR) is used for prenatal testing. We retrospectively assessed the frequency of both mosaic and nonmosaic conditions in a large-scale study and compared the clinical value of confirmatory cytogenetic analysis with QF-PCR and other screening tests.

Result: Of 6033 cases identified as abnormal conditions by sonography or protein marker screening tests, only 180 nonmosaic and 8 mosaic cases confirmed to be abnormal by confirmatory karyotyping test results. The cytogenetic analysis was correlated with other QF-PCR confirmatory test results for nonmosiac conditions but it was not comparable for mosaic cases.

Conclusion: The cytogenetic analyses were shown to have the greatest clinical value in revealing the various mosaic conditions. The QF-PCR test is shown to be a reliable confirmatory test for nonmosaic diseases but not for mosaicism, and the screening protein marker test can weakly indicate the presence of abnormal cell lines. Moreover, older mothers (>30 years) are at greater risk for developing mosaic ova.

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Prenatal disease; QF-PCR test; confirmatory tests; mosaicism; screening test; mosaic karyotype; nonmosaic

1. Background

Genetically prezygotic error in gonad cells can lead to abnormal conception and subsequently a prenatal disease. The induced genetic aberration carries out in only one cell line in a fetus whereas the error in the postzygotic period generates multiple-cell lines with different genotype in one individual fetus leading to mosaicism [1]. Both prezygotic and postzygotic errors present in the fetal chromosome, which can be detected by the prenatal tests (PTs). Moreover, mosaicism is not only generated normally in the ova of an older pregnant mother but also can be found in In Vitro Fertilization (IVF) blastocysts that adversely affect the pregnancy outcomes [2]. Nonmosaic genetic anomalies in fetus established when the entire genome is hampered by a meiotic chromosomal aberration within one cell line. However, occasionally mitotic error can be induced in multiple cell lines in a fetus with different chromosomal complex leading to mosaic syndromes. An error in cell division or non-disjunction and lag in anaphase is known to be the underlying causes of the mosaic condition in the ova of an older female. Generally,PTs are performed at various gestation age on pregnant female who are at risk for genetic disease, identified by screening tests such as sonographic image or multiple-protein marker test [3,4]

Amniocentesis and Chorionic Villus Sampling (CVS) are routine invasive PTs offered to a pregnant female with an increased risk of carrying a child with a nonmosaic genetic anomaly [5,6]. To detect a chromosomal abnormality, initially, the fetal cells are separated from their matrix, cultured in an appropriate medium, harvested, and then analyzed by karyotyping technique at the metaphasic stage [7,8]. In some clinics, molecular tests such as Fluorescent in Situ Hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA), array-Comparative Genomic Hybridization (a-CGH), and next-generation sequencing (NGS), are also performed along with karyotyping to avoid misdiagnosis and increase the sensitivity and accuracy of the PTs [9]. Recently, Quantitative Fluorescents polymerase-chain reaction (QF-PCR) have been introduced as a rapid screening molecular test for prenatal in

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which their results for the nonmosaic genetic test were compatible with the gold standard karyotype method [10,11].

Baby born with the mosaic condition generally tends to have a less severe symptom in comparison to the nonmosaic ones [12,13]. Mosaic conditions also create confusion and stress for the prenatal decision regarding the fate of their newborns. Chromosomal mosaicism is an interpretative issue in prenatal diagnosis because the severity level of symptom in the mosaic condition is related to the number and quality of chromosomal aberration in fetal tissues hence precise genotyping is crucial in diagnosis, management of the disorder, and in decision-making about the fate of the fetus [1,14]

The genetic error tends to occur more often in older ova hence comprehensive chromosomal screening test such as amniocentesis karyotype are suggested for ordinary pregnancy and Preimplantation Genetic Screening (PGS) should be performed for older female, who wish to undergo the IVF procedure for pregnancy. In addition to PGS, molecular tests such as NGS and aCGH have been used to identify embryos with chromosomal aneuploidy and mosaicism [2,15,16].

Placental mosaicism has been reported by invasive CVS at 9–12 weeks of gestation and also by noninvasive circulating fetal DNA (cfDNA) test in maternal plasma [17].

While QF-PCR and PCR-based MLPA test are performed on extracted fetal DNA, confirmatory tests like karyotyping and FISH are used to explore different structural and numerical chromosomal aberrations in the fetus. Although all prenatal confirmatory tests have high sensitivity and specificity only MLPA has shown to have 100% sensitivity and 100% specificity in the determining common aneuploidies in AF samples [18]

Mosaic aneuploidy can also cause birth defects with less deleterious effect and it can occur in forms of polysomy such as tetrasomy 18p [19].

The frequency of nonmosiac conditions are well known but the rate for the mosaic anomalies as well as their genotype, in a defined pregnant population remains unknown. Another factor that contributed to this study was the lack of comprehensive study involving prenatal diagnosis using the QF-PCR test in Iran. Nonetheless, chromosomal mosaicism or multiple-cell lines in the fetus have been recognized as a primary interpretative dilemma in genetic counseling as well as prenatal diagnosis. A number of studies have indicated that QF-PCR test results for nonmosaic disorders have been compatible with results obtained by karyotyping [10,20,21] but none of them found the test useful in identifying structural chromosomal aberration in the fetus.

This study aimed to analyze common genetic anomalies, including the mosaic and nonmosaic conditions, primarily through confirmatory karyotyping and the new rapid molecular QF-PCR tests to reconfirm the PTs result and compare these results with the screening tests such as serum protein marker test and sonography. These data can ultimately reveal the clinical value of confirmatory cytogenetic analysis along with QF-PCR test results and their correlation with other screening tests and also have important clinical indications and application for rare mosaic genetic imbalances as well as for genetic counseling.

To increase the sensitivity and specificity of the confirmatory tests in our lab, we performed major cytogenetic PTs such as amniocentesis, CVS, and occasionally cordocentesis along with a new molecular QF-PCR test.

Hence, in this study, the frequency of various types of mosaic and other chromosomal aneuploidies were determined using conventional cytogenetic with molecular QF-OCR test. Since common prenatal syndromes are primarily caused by chromosome X. Y, 13, 18, and 21, the molecular QF-PCR only detected the aneuploidy in these chromosomes, but standard cytogenetic was used to comprehensively describe all chromosomes in the fetal genome.

2. Methods

2.1. Sampling procedures and analysis

This was a retrospective monocentric study based on 6033 pregnant females who underwent invasive prenatal diagnosis by amniocentesis at 14th week, CVS at 12 Th week, cordocentesis at 10 Th week of gestations in the hospital between June 2010 to August 2015. Pregnant mothers with abnormal screening tests (e.g. serum protein markers or sonography) were selected for the invasive confirmatory test. To perform the confirmatory test AF sample, CVS, or fetal blood was obtained from each mother. The sampling procedure was performed base on the routine standard method, obtained fetal tissues divided into two portions for cytogenetic and molecular genetic analysis.

2.2. Cytogenetic procedure of prenatal samples

Fetal tissue samples were first separated from their matrix (blood, amniotic or placental fluids), grown in an appropriate culture medium and then cultured cells arrested at metaphase, harvested, treated with hypotonic solutions, washed, and lyzed on the slide to prepare a chromosome spread. Slides were stained with Giemsa to produce G-banding to elucidate the metaphase chromosomes and their karyotype.

2.3. Molecular QF-PCR procedure of prenatal samples

The fetal tissue sample was cleaned and their DNA was extracted by the specific extraction method designed for blood and tissue. The fetal DNA was

tested only for five major chromosomes that mainly involved in common prenatal syndromes. Two multiplex QF-PCR sets (S1 and S2) were used to conduct the initial analysis followed by final analysis via capillary electrophoresis. Moreover, four chromosome-specific extra marker sets (M21, M13, M18, and MXY) were used to finish the analysis (Table 1). Therefore, extracted DNA from fetal tissue was subjected to 5 classes of primers labeled with different colored fluorescent tags designated for chromosome X, Y, 13, 18, and 21. Hence, it has a limit of detection (LOD) for only five chromosomes. The DNA mixtures were placed into a PCR machine to amplify at 3–5 Short Tandem Repeats (STRs) found on the chromosome. The amount of fluorescence and size of copying DNA is measured, and ratios are presented graphically. DNA representing each allele in the sample was quantified by its peak using Gene Mapper Software version 4.0 (Applied Biosynthesis, CA, USA). The peak analysis was performed as follows: the peak with the height allele ratio e between 0.8 and 1.4 on at least two vivid markers were defined as normal. And markers with allele ratios between 1.4 and 1.8 or single peaks were reported as uninformative. But the presence of three alleles with an equal peak height ratio or with a ratio of ≤0.6 or ≥1.8 was documented as a trisomy. Hence, the number of peaks and height of each peak showed the number of copies of alleles at that region on the chromosome in the fetal DNA sample. QF-PCR can only detect both aneuploidy and polyploidy related to the aforementioned chromosomes.

2.4. Cytogenetic and molecular genetic analysis of prenatal samples

Initially, at least 20 G-banded metaphase spreads were analyzed karyotypically for each fetal sample, and when abnormal chromosome complex detected, then 30–60 more metaphase nuclei were included in our analysis to increase the confidence level and accuracy of the results statistically. Karyotype images were also provided for each case to document the result in each case. Molecular analysis of prenatal samples was performed based on sequencing data produced by QF-PCR. In a single molecular PTs, five sequencing markers were used for analyzing each chromosome 13, 18, 21, X, and Y to reveal quantitative aberration.

2.5. Molecular QF-PCR and karyotype result and patient information

Test with the abnormal or normal result was always reported to the parents, but genetic counseling is only given to the patient with mosaic condition case. To increase the accuracy (sensitivity and specificity) of PTs, the STRs results from QF-PCR were matched with each corresponding karyotype and analyzed for only the five most common chromosomes (X, Y, 21,13, and 18). We reported the common nonmosiac trisomies by both cytogenetic and QF-PCR but mosaic trisomies were detectable by karyotyping but not by QF-PCR test.

2.6. Comparison of screening tests with confirmatory tests

To determine the clinical value of the screening tests such as a quad or triple serum protein markers test and sonography images, each test result was compared to golden standard karyotype as well as QF PCR results. Mosaic conditions revealed by the cytogenetic analysis were also matched with QF PCR data and other screening tests to elucidate the discrepancy between these tests.

2.7. Control population

Patients with normal fetal karyotype, QF-PCR, and screening tests were used as a control population, and for comparison to abnormal cases.

2.8. Statistical analysis

All quantitative data were analyzed by statistical software (prism) and described by descriptive statistical measures, including mean, median, and range. For quantitative analysis, we used a 95% confidence level and P values less than 0.05 were statistically considered significant.

Table 1. Chromosome aneuploidy detection by QF-PCR using different fluorochromes.

Markers	Location in chromosome	Dye	known alleles (bp)
DXYS218	Xp22.32 Yp11.3	PET	266–270-274-278-282
D21S1414	21q21	6-Fam	342–346-350-352-354-356-358
D21S1008	21q22.1	6-Fam	204–208-212-216-220
D18S535	18q12.2	NED	126–130-134-138-142-146-148-152-156
D13S631	13q31-32	VIC	192–196-200-204-208
SBMA	Xq11.2-Xq12	VIC	178–181-184-187-190-193-196,199
D21S1437	21q21.1	VIC	128–132-136-140-144
D21S1435	21q21	PET	142–160-164-168-172-176-180-184-188

2.9. Ethical aspect

This research was approved by the ethical committee of the Iran University of Medical Science (IR.IUMS.REC.1396.05.32).

3. Results

This was a large-scale prenatal study that was conducted from June 2010 till August 2015. We performed 6033 cases of amniocentesis, CVS, or cordocentesis in a well-defined population of pregnant females with problematic screening tests such as imaging and quad or triple protein markers. Of 6033 cases with suspicious protein marker tests, only 188 (3.11%) were identified as abnormal conditions that were divided into two categories of nonmosaic and mosaic cases. The nonmosaic conditions including; 75 (39.89%) Down's syndrome, 42 (22.34%) Edward syndrome, 37 (19.68%) patau syndrome, 11 (5.85%) super female, 3 (1.59%) Turner syndrome, 2 (1.06%) Jacob, and 2 (1.06%) nonmosaic chromosomal aberration with translocation and only 8 (4.25%) cases of mosaic conditions of various types. The nonmosaic syndrome cases were identified by standard cytogenetic karyotyping as well as the QF-PCR test successfully. Mosaic cases were detected successfully through conventional karyotyping, but the QF-PCR test failed to reveal the mosaic cell line and structural anomalies for their designated chromosomes (Table 2).

QF-PCR tests in all 8 mosaic cases (0.13%) could not detect the abnormal cell lines and STRs in all five designated chromosomes showed only normal chromosome or identified the mosaic chromosome as a no mosaic (Table 2), but in overwhelming cases of nonmosaic cases aneuploidies of chromosome 13,18,21, X, and Y were detected by QF-PCR (data are not shown).

Moreover, of the 8 mosaic cases by karyotype, 5 had an autosomal mosaic condition, 2 sex mosaic condition, and one marker chromosome mosaicism (Figure 1).

Karyotype analysis indicated that mosaic genotypes consisted of four autosomal aneuploidies identified as trisomy 21 and one as monosomy 21. Also, two sex mosaic aneuploidies were detected, in which, one was trisomy of XXY and one monosomy of X chromosome. There was also a new case of undefined marker chromosome mosaicism. Nonetheless,

Table 2. Distribution of nonmosiac genetic in tested population.

Genetic Anomalies	Types of Anomalies	Karyotype	QF-PCR
75 (39.89%)	Down syndrome	t + 21	Abnormal
42 (22.34%)	Edward syndrome	t + 18	Abnormal
37 (19.68%)	Patau syndrome	t + 13	Abnormal
11 (5.85%)	Super female	XXX	Abnormal
3 (1.60%)	Turner's syndrome	X	Abnormal
8 (4.25%)	Klinefelter syndrome	XXY	Abnormal
2 (1.06%)	Jacob syndrome	XYY	Abnormal
1 (0.53%)	Translocation	Between X and Y chromosome	Normal
1 (0.53%)	Translocation	Between 6/15	Normal
8 (4.25%)	Mosaic	Various karyotype	Normal

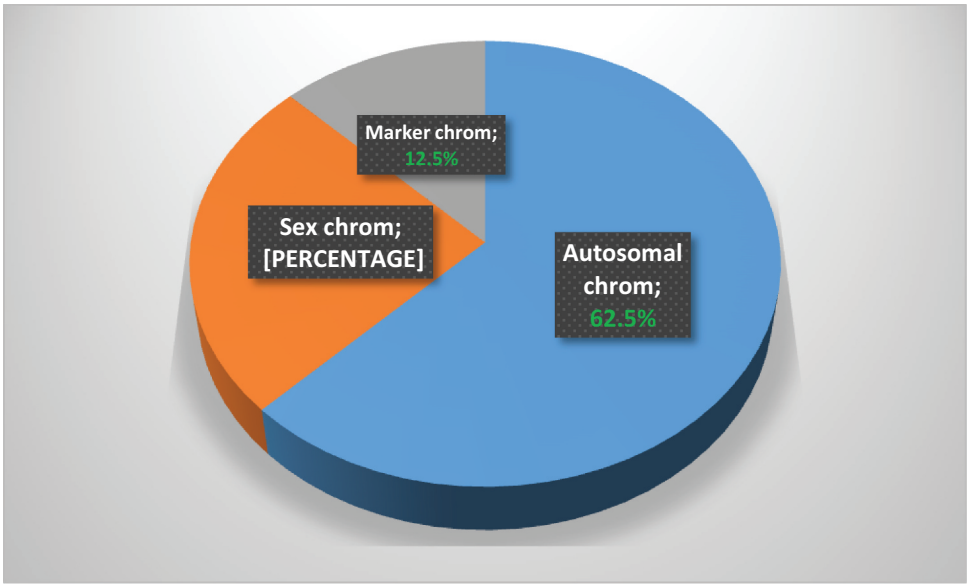


Figure 1. Mosaic proportion of various chromosomal aberrations.

the imbalance mosaic conditions were distributed equally among both sexes as it is illustrated in Table 3.

Among the mosaic cases, we found one new case of mosaicism with monosomy of chromosome 21 for the first time and one case of a mosaic marker chromosome in which the nature of the marker chromosome could not be determined.

3.1. Distributions of chromosomal aberration in mosaic cell lines

Of four trisomy 21 mosaic conditions, case-1 had approximately 4% trisomy 21 and 96% normal metaphases with female gender and 2nd male gender and in third 92% trisomy 21 and the rest normal metaphase with also male gender, and in the case-4, 71% trisomy 21 and 29% were normal metaphase with the male gender.

Case 5 was a mosaic of chromosome 21 in which it had close to 4% of monosomy of 21 and 96% normal chromosomal. Case 6 was a sex chromosomal mosaic in which 90% of metaphase spread had trisomy of sex chromosome XXY and only 10%normal cells. Case −8 was also a sex mosaic condition in which 40% of metaphases were monosomy of X-chromosome (X) and 60% normal 46 XX (Figure 2). The case-7 was an unidentified marker chromosome with 30% marker chromosome aneuploidy and 70% normal female chromosome (Table 3).

3.2. QF-PCR test results for mosaic and nonmosaic conditions

QF-PCR was also carried out along with conventional cytogenetic karyotyping to detect the mosaic, but it was not successful because it was not sensitive or specific enough to identify chromosomal aberration in multiple cell lines. The STRs markers in QF-PCR were only identified normal cell lines in mosaic conditions, but unable to detect genetic aberrations in the abnormal cell line. Nevertheless, QF-PCR molecular test was shown to be helpful in the majority of AF samples in identifying nonmosaic prenatal anomalies such as trisomy 21, monosomy 21 and, trisomy 13, 18 and sex aneuploidies.

3.3. Confirmatory test versus screening test for risk indication

Screening quad protein marker tests were not strongly correlated with conventional karyotype nor with QF-PCR test. They only warrant the presence of a genetic

Table 3. The frequency of mosaic karyotype.

Case Condition	Mosaic Chromosome	Karyotype
Case-1-Autosomal	Trisomy 21	46,XX[96%]/47,XX,+21[4%]
Case-2-Autosomal	Trisomy 21	46,XY[10%]/47,XY,+21[90%]
Case-3-Autosomal	Trisomy 21	46,XY[8%]/47,XY,+21[92%]
Case −4-Autosomal	Trisomy 21	46,XY[29%]/47,XY,+21[71%]
Case-5-Autosomal	Monosomy 21	46,XX[96%]/45,XX,-21[4%]
Case-6-Sex	Trisomy Sex Chromosome	46,XY[10%]/47,XXY[90%]
Case-7-Marker	Marker Chromosome	46,XX[70%]/47,XX,+mar[30%]
Case-8-Sex	Monosomy Sex Chromosome	46,XX[60%]/45,X [40%]

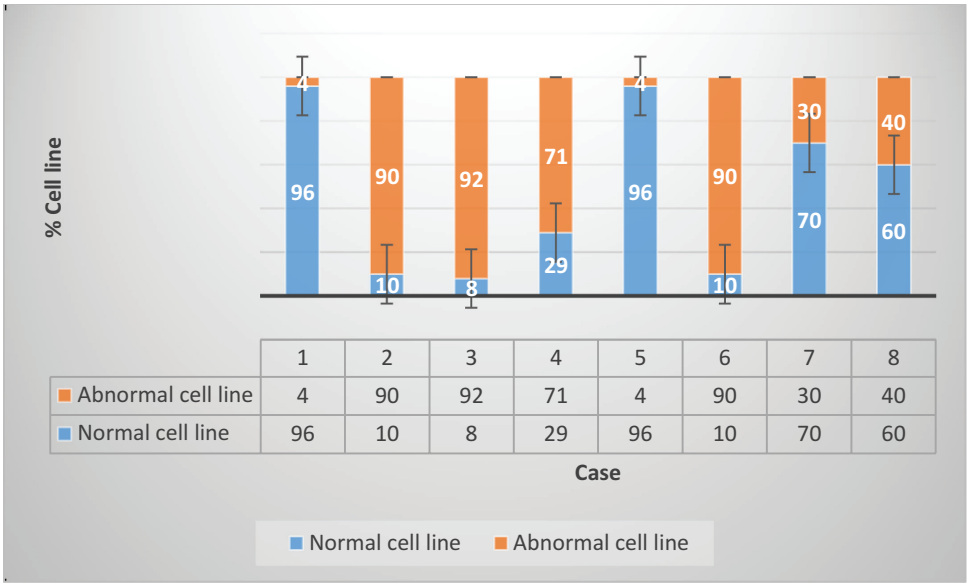


Figure 2. Distribution of normal cell line vs. abnormal cell line in Mosaic conditions.

anomaly in chromosome 21, but could not produce any precise rate for chromosomal aberration nor could indicate different mosaic cell lines (Table 4).

For instance, in case 1 the screening protein marker index (PMI) for risk indication of chromosome 21 was 2% (trisomy 21 = 1/54) and the karyotype mosaic ratio (KMR) was 2/51 or 3.85%. Case 2 had KMR = 90% with no screening test. In case 3 the PMI of t21 was 1/8 or 12.5% with KMR = 96%. The PMI t21 for case 4 was 1/72 or 1.5% with KMR = 71%. In case 5 the PMI t21 was 1/203 or 0.5%, but in reality, it was a mosaic of monosomy 21 with KMR = 3.64%. Case 6 was a mosaic of sex chromosome (XXY) in which its KMR was 90% with the PMI t21 = 1/31 or 3.23% with no indication of risk for sex chromosome in the screening test. Case 8 was also a mosaic of sex chromosome for Turner syndrome with PMI t21 = 1/170 or 0.6% without any indication of risk for turner syndrome in the screening test and its KMR = 40%. Case 7 was mosaic of marker chromosome in which its PMI t21 = 1/120 or 0.83% and its marker KMR = 30% (Figure 3).

Table 4 also displays that mosaic aneuploidy occurred primarily in the older mother with an age range of 32–40 years (>30 years old).

3.4. Screening test and image analysis of mosaic risk indication

Although some abnormalities detected by confirmatory tests for nonmosaic conditions matched with risk indication in sonography image, but no anatomical anomaly was detected for the mosaic condition in image analysis (Table 3).

4. Discussion

Common prenatal diseases such as Down's, Edward, Patau, Klinefelter, Jacob, and Turner syndromes usually occur with a specific chromosomal aneuploidy in a single-cell line that is generated by a meiotic error, but occasionally if error induced in postzygotic cell division, then it may lead to production of the normal cell line admixed with one or two abnormal cell lines for a particular chromosomal aberration, leading to a mosaic condition. Of 188 cases of genetic anomalies in our study, only 4.25% were mosaicism indicating that it is a rare event in prenatal disorders (Table 2). Moreover, mosaicisms with autosomal chromosomes were more prevalent (63%) than other types.

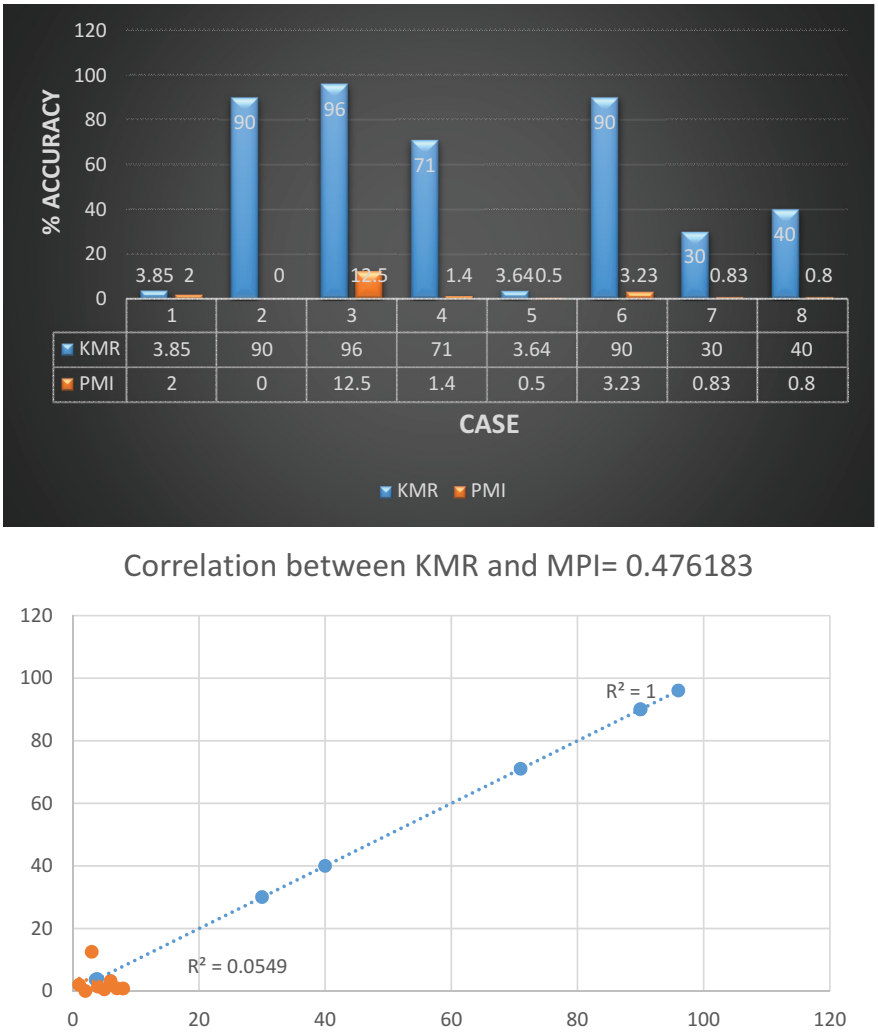
Nondisjunction and delayed anaphase are the main mechanisms that lead to error in mitosis and meiosis for mosaic and nonmosaic disorders, respectively. It is well known that both types of genetic errors induced in aged gonad cells [1,7,16]. Our current results showed mosaic conditions predominately occurred in older pregnant mothers (>30 years old) as it is depicted in Table 4. The proportion of abnormal cell lines in mosaicism influences the severity of disease in the newborn baby.

The genetic errors in our mosaic conditions result in various rates of abnormal cells as it is shown in Table 3, and consisted of six mosaic cases (2–4, and 6–8) that harbor the highest rate (30–92%) of abnormal cell lines but other cases (1, 5) had the lowest rate (4%) of abnormal cell lines.

In recent years complementary and confirmatory molecular tests have made improvements in the prenatal diagnosis of both mosaic and nonmosaic conditions. Conventional cytogenetic PTs though considered to be the best comprehensive method to identify genetic anomalies in the fetus, but it requires strict aseptic culturing condition along with rigid karyotyping. Molecular tests, in particular; MLPA, FISH, QF-PCR, chromosomal microarrays, and NGS not only bypass the need for culturing the specimens, but also produce results within a few working days thereby reducing the waiting periods and anxiety for the parents and increasing the confidence level of result. These technologies are also useful in the detection of various nonmosaic prenatal diseases

Table 4. Comparison of mosaic karyotype and screening test.

Case number	Mosaic chromosome	Karyotype	Protein marker	Gestation week	Female age	Image Analysis
Case-1-Autosomal	Trisomy 21	46,XX[50]/47,XX,+21 [2]	t21 = 1/24	18	38	Normal
Case-2-Autosomal	Trisomy 21	46,XY[2]/47,XY,+21 [18]	____	20	38	Normal
Case-3-Autosomal	Trisomy 21	46,XY[2]/47,XY, +21[48]	t21 = 1/8 t13 = 1/ 11	18	40	Normal
Case-4-Autosomal	Trisomy 21	46,XY[9]/47,XY,+21 [22]	t21 = 1/72	16	38	Normal
Case-5-Autosomal	Monosomy 21	46,XX[53]/45,XX,-21 [2]	t21 = 1/203	18	38	Normal
Case-6-Sex	Trisomy Sex Chromosome	46,XY[5]/47,XXY[45]	t21 = 1/ 31	16	32	Normal
Case-7-Marker	Marker Chromosome	46,XX[14]/47,XX,+mar [6]	t21 = 1/120	18	35	Normal
Case-8-Sex	Monosomy Sex Chromosome	46,XX[30]/45,X [20]	t21 = 1/170	16	33	Normal



	1	2	3	4	5	6	7	8
KMR	3.85	90	96	71	3.64	90	30	40
PMI	2	0	12.5	1.4	0.5	3.23	0.83	0.8

Figure 3. Karyotype Mosaic Ratio (KMR) vs. marker protein indices (PMI).

in low-frequency cell lines but have shown to be less effective in identifying various chromosomal cell lines found in mosaicism [1,22]. In our study, we also found that QF-PCR test results for mosaic cases were not compatible with the karyotype results as it is shown in Table 2, but it correlates significantly ($p < 0.05$) with nonmosaic karyotype results (Tables 2 and 3). In agreement to our result, study by 21 [21], reported that four prenatal cases identified by karyotyping as mosaicism, in the QF-PCR test were detected as nonmosaic trisomies. Conversely, the conventional karyotyping method revealed all possible cell lines that may occur in a single fetus, in our study (Table 4) and hence considered a golden standard procedure for identifying the various mosaic cell lines.

Moreover, AF mosaicism occurred in various levels with the observations of single, two, or more abnormal cell lines in a culture, that can be readily detected by conventional karyotyping, whereas mosaicism detected by CVS analysis is not very sensitive or accurate and has to be reconfirmed by amniocentesis [1]. The diagnostic accuracy of the QF-PCR test in our study was not comparable to karyotyping for the mosaic conditions, but it was comparable to nonmosaic PTs (CI = 0.999–1.0).

It is known that the pathogenicity of mosaic condition coincides with the proportion of abnormal cell lines, and the degree of mosaicism influences the severity of symptom produced in the fetus [2,6,8,12,13,18]. In our study, cases 2–4, and 6–8 had more abnormal cells than normal; hence, they would have more symptoms than those (cases 1 and 5) with fewer abnormal cell lines. In this study, various cell lines in mosaicism were shown (Table 3), and the ratio of abnormal cells verses normal cell were calculated, as KMR for symptom analysis for genetic counseling as well as risk indication. We found a very weak correlation between confirmatory test results, including karyotyping or QF-PCR test with screening tests such as serum protein marker test as well as ultrasound image analysis. In our study, conventional karyotype seems to produce the best diagnosis and indication risk alarm for various mosaic or nonmosiac prenatal diseases, whereas QF-PCR due to lack of detecting abnormal cell line in mixed cells had no risk warning indication for mosaic

anomalies but it relatively produces valuable diagnostic information for five STR chromosomes X, Y, 13, 18, and 21 in nonmosaic diseases. Nevertheless, screening protein marker test or sonography though weakly indicated the risk but unable to verify various cell lines as it is illustrated in Table 4.

Here, we report 0.13% mosaic conditions in more than 6000 AF, CVS, and fetal cord blood samples within all diagnosed genetic anomalies. We calculated the autosomal mosaic to be 2.5-fold higher than sex chromosome mosaicism.

Various obstacles such as fetal-placental discrepancies and the uniparental disomy (UPD) condition can lead to erroneous diagnosis; hence, CVS with two short- and long-term cultures was established to reduce the risk of misdiagnosis. Additionally, confirmatory amniocentesis in some situations was necessary to increase the confidence in the diagnosis of fetal chromosome status [23]. The amniocentesis PTs comprised most of our analysis in this study because CAs can be easily detected by a karyotype method [11,24].

Mosaicism can occur at two levels of chromosome and gene mutation. Moreover, mosaic mutations are found in the parental germ lining as sex mosaicism. More than 100 Mendelian disorders are associated with both parental germline mosaic mutation and somatic mosaic [25,26]. Worldwide de novo mutation data show that mutational mosaicism augmented in both paternal and maternal cells with an increase in age [27]. Overall mosaic aneuploidy in this large-scale study was evenly distributed in both sexes with no significant differences ($P > 0.05$) (Table 3). Moreover, mosaic conditions were detected in older pregnant females as it is reported in Table 4.

The QF-PCR test was limited to identifying aneuploidy of only five chromosomes (x, y, 13, 18, and 21); hence, it could not reveal other chromosomal aberrations. Nonetheless, the QF -PCR test results were shown to have great sensitivity (92.6%) for nonmosaic anomalies with a single-cell line, but could not detect variations of cell lines (Table 2). This is probably due to the lack of sensitivity and specificity of markers in the test, to distinguish combined normal and abnormal cell lines. We, therefore, suggest other molecular tests such as MLPA or FISH with higher sensitivity and specificity to be used as confirmatory tests of mosaicism rather than QF-PCR.

Ideally, mosaicism should be diagnosed by screening a large number of karyotype metaphases in order to decrease the error and produce a statistically more confident result but we were also limited in the quantity of metaphase. Our study, however, indicated a wide variety of frequencies of abnormal cells within eight cases of the identified mosaic condition. Hence, other complementary PTs should be explored to compensate for the shortcoming in metaphases. Interestingly, we found one new case of marker chromosome mosaicism with ambiguous morphology that generated a more challenging situation for genetic counseling than those with known characterized morphology. We also had a new mosaic case of monosomy of 21 with an unknown fate for genetic counseling (Tables 3 and 4).

4.1. Ultrasound images are important for screening nonmosaic conditions

The ultrasound image analyses did not show any physical anomalies in all mosaic cases (table-4). This could be due to a combined effect of both normal and abnormal cell lines in the fetus development or subtle abnormal anatomical feature in mosaic anomalies. Improved ultrasound devices and analytical experience enable the obstetricians to characterize and identify abnormal phenotype in various mosaics [19]. The image analysis revealed physical anomalies in overwhelming nonmosiac conditions.

4.2. Confirmatory karyotype versus multiple protein marker tests

The karyotype test results confirmed only 3.11% of all positive protein marker test. Hence, the risk indiction factor difference between karyotyping KMRs and PMIs for protein marker test was very significant ($p = 0.05$), and in sex mosaic cases, protein marker tests did not even indicate a warning for sex chromosome aneuploidy and instead, it showed aberration for chromosome 21. Therefore, protein marker tests can only poorly signal autosomal aneuploidy. Although positive mosaicism is weakly defined by marker protein tests, but negative marker protein results were useful in eliminating unnecessary invasive PT.

Thus, conventional karyotyping showed a robust detection power for screening chromosomal aberrations in both mosaic and nonmosaic conditions, whereas the molecular QF-PCR could only be useful for nonmosaic anomalies. Therefore, QF-PCR molecular test in the present study shown to be useful in identifying chromosomal aberration in one cell-line syndrome but not in multiple cell lines as is indicated in Table 2.

The present study showed that diagnostic reference values of mosaicism differed significantly between confirmatory karyotype and protein marker screening test ($p < 0.05$). This discrepancy could be due to the fact that the normal cell-line effect would overcome the abnormal cell line thereby generating normal protein marker results.

Based on obtained data, we came to the conclusion that mosaicism generated by genetic error, is a rare event that occurs mostly in somatic chromosomes with varied abnormal cell lines, and older mothers (>30 years old) are more prone to this condition.

5. Conclusion

The cytogenetic analysis showed the greatest clinical value in revealing the various mosaic conditions. The QF-PCR test shown to be a reliable confirmatory test for nonmosaic diseases but not for mosaicism or structural aberration in fetal chromosomes. Image analysis and screening protein marker tests can weakly indicate the presence of abnormal cell lines. Moreover, older mothers (>30 years old) are at greater risk for developing mosaic ova.

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