ORIGINAL ARTICLE

Genetic Evaluation of Spontaneous Miscarriages and Couples through Conventional and Modern Diagnostic Tools

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Abstract

Background: Genetic causes of miscarriages have been extensively reported and studied in almost half of the first-trimester pregnancy losses, for that reason genetic investigations of the products of conceptions (POCs) are crucial to help identify possible genetic etiology. This information is helpful for the parents as it provides them with more acceptance of the loss and helps them recognize possible recurrence risks.

Objective: This study aims to evaluate Quantitative Fluorescent-PCR (QF-PCR) for chromosomal aneuploidies detection in product of conception (POCs) as an alternate to conventional karyotyping and to explore potential parental chromosomal abnormalities causing recurrent spontaneous miscarriages.

Methodology: A total of seventy-six products of conceptions (POCs) were obtained from the Gynecology department of Civil Hospital, Karachi. Further blood samples from forty-five couples were also collected in addition to ninety-three maternal blood samples. All of these samples were subjected to amplify short tandem repeat (STR) for common chromosomal aneuploidies (13, 18, and 21). Real-time QF-PCR was performed on 76 POCs on Sansure Biotech Inc, Changsha, China, using primers D13S631 and D13S634 for chromosome 13, D18S386 and D18S535 for chromosome 18, and D21S1411 and D21S1414 for chromosome 21. Parental blood samples were examined by both QF-PCR and karyotyping for cross-checking with their POC. Marker on Chromosome 16 was used as an internal control for the amplification of each reaction. Statistical analysis was done by using SPSS version 24.

Results: QF-PCR analysis revealed chromosomal aneuploidies among twenty-nine POCs with the highest number of trisomy 13 followed by trisomy 21 and then trisomy 18 while all the parental samples were normal. Nine cases of chromosomal abnormalities were ruled out through karyotyping from parental blood samples further, a Robertsonian ttranslocation involving chromosome 21 was observed through conventional cytogenetics and interestingly the corresponding POC was found to have trisomy 21.

Conclusion: This study's findings endorsed QF-PCR as an efficient technique to detect chromosomal aneuploidies. Advantages of QF-PCR over conventional karyotyping is that it is less laborious, has lesser turnaround time, is more economical, and has a low failure rate.

Keywords: *Miscarriage, chromosomal aneuploidies, chromosomal abnormalities, numerical aberration, short tandem repeat.*

INTRODUCTION

Spontaneous miscarriage (SM) is the most common and perplexing pregnancy complication due to the possibility of multiple factors simultaneously. In the first trimester, most of the SM occurs due to the non-disjunction of chromosomes [1]; with approximately 51.9% of trisomies, 18.8% polyploidies, and 15.2% of monosomies due to the non-disjunction [2-4]. Women and their spouses suffer from depression and self-blame after having a spontaneous miscarriage, and this guilt increases many times when they have recurrent spontaneous miscarriages. Timely recognition of the etiology of this irreparable loss is crucial for better genetic counseling of the families for risk assessment in upcoming pregnancies and to plan [2, 5, 6]. In many countries, conventional cytogenetic analysis has been performed in prenatal diagnosis for centuries, even in the case of first spontaneous pregnancy loss [7, 8]. However, the cumbersome process of cell culturing, prolonged reporting time, and high rate of culture failure (up to 40%) demand more robust molecular techniques [9, 10]. Quantitative Fluorescent-PCR (QF-PCR) has already been introduced in many reputable molecular diagnostic centers in the west. Several researchers have reported QF-PCR as a reliable technique for aneuploidy detection in the product of conceptions (POCs) through short tandem repeats (STRs) amplification [6, 11]. Even though the affordability of the genetic analyzer is still under debate [12, 15], this study was designed to evaluate the efficacy of the rapid screening technique QF-PCR on a real-time PCR machine instead of using a genetic analyzer. Fluorescently labeled STR markers

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were used to detect common aneuploidies in contrast to cytogenetics. Further, this study highlighted the possible parental chromosomal abnormalities liable to cause recurrent spontaneous miscarriages.

METHODS

The study was conducted at the Department of Obstetrics and Gynaecology Civil Hospital, Dow University of Health Sciences Karachi, after approval from the Institutional Ethical Review Board with reference number BASR/No/02475/Sc. Written informed consent was also obtained from each of the participants after thoroughly explaining the purpose of the study in their understandable language. A sample size of 139 cases was calculated through epi info software version 7.2.4., with the expected frequency of spontaneous miscarriages at 10% in all known pregnancies at a 95% of confidence level and 5% margin of error [1]. During the study period between January 2016 and November 2018, one hundred and eighty-three cases were recruited, consisting of blood samples of forty-five couples and ninety-three maternal blood samples. Seventy-six products of conceptions (POCs) or fetal muscle samples (8 to 12 weeks of gestation) were also obtained from the recruited couples where available and amplified through QF-PCR. Parental blood samples from forty-five couples were analyzed with both QF-PCR and conventional cytogenetics to compare the two techniques (**Fig. 1**). Standard protocols were followed, and all possible quality control measures were taken during the selection of the tissues from the abort uses [16].

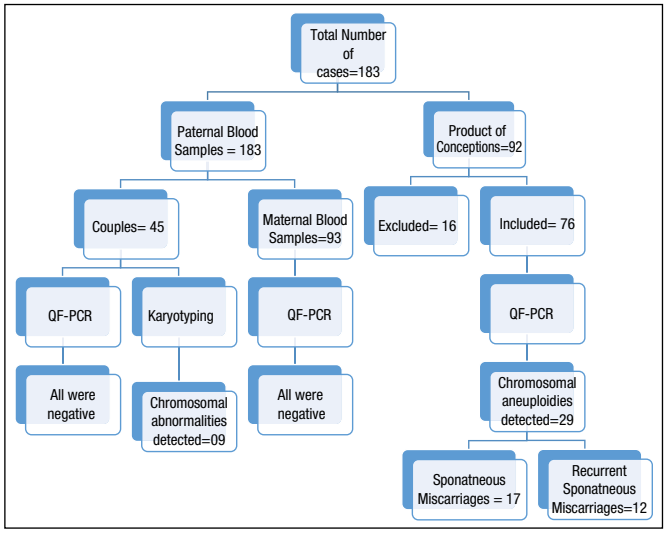

Fig. (1): Methodology and result findings.

QF-PCR was performed on real-time PCR Sansure Biotech Inc, Changsha, China, using reported STR (short tandem repeat) markers (D13S631 and D13S634 for chromosome 13, D18S386 and D18S535 for chromosome 18, and D21S1411 and D21S1414 for chromosome 21). Chromosome 16 was used as a reference marker for each reaction with primers F-5'-CAGGCTGCGATGAGAACATA-'3, R-5'-CTAGGCAGGAAAGCGTCTTG-'3. Data acquisition was performed during the annealing/extension step. The relative quantification was done using the test marker's CT value (cycle threshold) and the reference marker (chromosome 16; GenBank: NG_000006.1, positions 23261–23355). The difference between each reference marker and test marker was mentioned as ΔCT. Each reaction contained positive and negative controls for the validation of the experiment. ΔΔCT value was calculated by subtracting the ΔCT of negative to each ΔCT of the tested marker to obtain 2^-ΔΔCT had already been performed and reported by Sanguansermsri *et al.* in 2014 [10]. Karyotyping was performed only on parental blood samples obtained from the couples; for this purpose, 20 random metaphases were assessed using Ikaros and Isis metasystem. The International System for Human Cytogenetic Nomenclature was used as a reference for karyotyping [17]. All the biological samples collected for this research were properly disposed of as per institutional protocol in an ethically responsible and environmentally friendly way.

Data were analysed using SPSS version 21. Categorical variables were expressed as frequency and percentage.

RESULTS

This was a single-center study; all recruited families were from a low socioeconomic background with maximum primary school education. A broad spectrum of ethnicity was observed in the sample, including Sindhi, Punjabi, Saraiki, Pushto, and Urdu speaking (**Table 1**). Moreover, a high rate of miscarriages was found among younger women (>30 years of age) though the p-value was non-significant (0.70). Short tandem repeats (STRs) were successfully amplified through real-time QF-PCR on all

Table 1: Demographics.

Demographics	Frequency	Percentage
Consanguinity		
No. of consanguineous couples	95	67.9
No. of non-consanguineous couples	45	32.1
Types of miscarriages		
No. of spontaneous miscarriages	88	62.9
No. of recurrent spontaneous miscarriages	52	37.1
Gestational age		
1st-trimester miscarriages	113	80.7
2nd-trimester miscarriages	27	19.3
Total no. of cases = 140		

Table 2: Numerical Chromosomal Aberration Identified by QF-PCR.

Aneuploidies in POCs	No. of Cases	Percentage
Trisomy 13	12	15.78
Trisomy 18	4	5.26
Trisomy 21	10	13.5
Trisomy 13, 18	1	1.31
Trisomy 13, 21	2	2.63
Total no. of aneuploidies	29	38.15
Total no. of POCs= 76		

Table 3: The ratio of Abnormal Karyotype of Couples concerning Genders.

Gender	Karyotypes	Chromosomal Abnormalities
Male	46,XY(07)/47,XXY(09) 46,XY(08)/47,XXY(05) 46,XY,15PS+(15) 45,XY,der,(14:21),(q10;q10)	Trisomy Trisomy Minor Structural Abnormality Robertsonian Translocation
Female	46,XX,15PS+(19) 46,XX,inv(9)(p11q13)(17) 46,XX,inv(9)(p12q13)(15) 46,XX,t(11;22)(q23;q11.2) 47,XX,+mar	Minor Structural Abnormality Inversion Inversion Reciprocal Translocation Marker
Total no. of abnormal karyotypes= 09		

Table 4: Comparison of Abnormal Parental Samples with POCs.

Abnormal Karyotype of Parental Sample	QF-PCR of POCs
46,XY(07)/47,XXY(09)	Normal
46,XY(08)/47,XXY(05)	Normal
46,XY,15PS+(15)	Normal
45,XY,der,(14:21),(q10;q10)	Trisomy 21
46,XX,15PS+(19)	Normal
46,XX,inv(9)(p11q13)(17)	Normal
46,XX,inv(9)(p12q13)(15)	Normal
46,XX,t(11;22)(q23;q11.2)	Normal
47,XX,+mar	Normal

76 products of conceptions (POCs) and 183 parental blood samples (45 couples and 93 maternal blood samples). A total of 29 aneuploidies were detected in POCs, while all the corresponding parental samples were found normal for the tested markers (**Table 2**). Successful cell cultures were obtained from peripheral blood samples of forty-five couples, and karyotyping recognized nine chromosomal abnormalities. Abnormal karyotype was found with a ratio of 1:1.25 in male and female carriers, nearly the same in both genders and no significant association was found with any gender (**Table 3**). Remarkably, in a POC of a male carrier with Robertsonian translocation trisomy, 21 was identified through QF-PCR (**Table 4**).

DISCUSSION

Regardless of significant improvement in prenatal care, women still suffer from the loss of a pregnancy or neonatal death. Spontaneous miscarriage (SM) has always been distressing for the couple, similar to stillbirth. Proper counseling and risk assessment are crucial for improving their mental health and managing subsequent pregnancies.

Advanced maternal age is known to be a cause of increased chromosomal abnormalities in fetuses due to non-disjunction during the first meiotic event. It leads to chromosomal aberrations causing spontaneous miscarriage (SM) [1, 2]. Similarly Shamshad and Priyadarsini 2016, reported that rate of miscarriages is directly proportional to the age of women [18]. However, in our study, most of the miscarriages were found in younger women between the age of 26 and 30 years. Possible reasons for this finding are the small sample size of the study and that women usually conceive at a younger age in our population.

Investigation of the product of conceptions (POC) is crucial as most genetic abnormalities that cause spontaneous pregnancy loss can only be found in POCs. Therefore, genetic causes of SM are still underestimated in the live population. Chromosomal screening through conventional cytogenetic methods is extensively performed in prenatal diagnostic setups, even though the high rate of culture failure due to improper technique or poor handling makes it a cumbersome procedure. Further, couples have to wait for at least 15 days, which dramatically increases their anxiety, especially when time is construed in the decision-making of the pregnancy [6]. In this study, a newer technique, QF-PCR, was performed on all the samples (parental blood samples and POCs) and found 29 aneuploidies among POCs, while all parental blood samples were found normal, which were expected to be normal.

On the other hand, the conventional cytogenetic method found nine cases of abnormal karyotypes. Of these two couples had 46, XY/XXY mosaicism in male partners, which has already been reported in many studies as the liable cause of miscarriage [19-22]. Two couples had increased length of chromosome 15p arm; in one case, the male was the carrier and the other female. Previously, increased length of chromosomes was reported to cause non-disjunction, which can result in pregnancy loss [23]. Two couples had chromosome 9 inversion in female partners; chromosome 9 is well known for its heteromorphic nature, which can cause spontaneous pregnancy loss [24]. One couple had a small supernumerary marker chromosome in the female partner. These supernumerary chromosomes are difficult to illustrate but are known to cause SM [14, 25]. One couple had a balanced reciprocal translocation 46, XX,t (11;22) (q23;q11.2) in the female partner. One more reciprocal translocation was found in males with a Robertsonian translocation 45, XY, der, (14:21), (q10; q10). This particular couple with Robertsonian translocation suffered seven pregnancy losses, and the POC from this couple was noted to have trisomy 21. Balanced translocation has even more tendency to cause pregnancy losses due to the hidden partial trisomy, although balanced translocation carriers are normal [26].

Out of the nine carriers of abnormal karyotype, only one POC was found to have aneuploidy. More primers need to be designed to investigate POCs through QF-PCR comprehensively. Similar results were shared by a recent study in which both techniques were assessed parallel and supported the QF-PCR as an efficient method [27]. QF-PCR was declared a consistent technique at affordable cost by using the real-time PCR machine instead of an expensive genetic analyzer, which was found to be excellent as it consumes less time and is capable of detecting multiple aneuploidies within 24-48h

[28, 29]. Sanguansermsri *et al.* in 2014, stated the QF-PCR's efficacy in aneuploidy screening by calculating CT values [10]. Similar to other techniques, QF-PCR also has some limitations, as QF-PCR is not practical for detecting structural chromosomal abnormalities; contrary to this, karyotyping has been known for identifying structural chromosomal abnormalities since the 1980s. However, the prolonged reporting time influences patients and their families to choose QF-PCR [2].

In many western countries, QF-PCR has already been initiated as the primary test among a routine panel of prenatal diagnosis [1, 6, 8-9, 13]. Many scientific researchers have announced the practicability and precision of QF-PCR; in 2017, a researcher reported 100% accurate results; in another study, 98.05% of the findings were consistent with conventional cytogenetics [8, 9]. In the same way, Tekcan *et al.* 2014 revealed 100% of concordance of QF-PCR with karyotype [27]. As reported in different articles, QF-PCR has verified itself as an adjunct technique to conventional karyotyping [30]. QF-PCR is a cost effective less laborious as it does not necessitate cell culturing, so it has a lesser turnaround time and can be done with a small amount of DNA through automation [31]. It has a high success rate of up to 95% in terms of aneuploidy detection [32-35].

CONCLUSION

Chromosomal evaluation of the product of conceptions (POCs) is vital as it may provide a possible explanation of the causes of miscarriage. This study revealed, Quantitative Fluorescent-PCR (QF-PCR) as a robust technique for detecting common chromosomal aneuploidies; though conventional karyotyping remains as the standard gold as it also detects chromosomal structural abnormalities. Findings of this study and other previously reported studies have endorsed that QF-PCR could be performed as a standalone technique for aneuploidy detection. In the west, Non-Invasive Prenatal Testing (NIPT) has now been adopted to screen for fetal aneuploidy detection and Constitutional Chromosomal Microarray (Array CGH) is used for comprehensive chromosomal studies. However, QF-PCR could be an alternative method until these techniques become readily available in resource-poor countries like Pakistan.-

ETHICS APPROVAL

The study was conducted at the Department of Obstetrics and Gynaecology Civil Hospital, Dow University of Health Sciences Karachi, after approval from the Institutional Ethical Review Board with reference number BASR/ No/02475/Sc. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the Helsinki declaration.

CONSENT FOR PUBLICATION

The written consent forms were signed by the participants.

AVAILABILITY OF DATA AND MATERIALS

Data used to calculate the results are available and can be provided by the corresponding author upon request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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None.

AUTHORS' CONTRIBUTION

The first and the second authors conceptualized the study, searched the literature, and participate in writing the manuscript. The second and third authors designed the study protocol. The fourth and fifth authors received ethical permission and facilitate data collection. The seventh author critically analyzed the data and approved the final version for publication.

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