Frequency of Chromosomal Abnormalities in Products of Conception: A Retrospective, Large-scale, Single-center study

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ABSTRACT

Background: The cause of miscarriage is successfully elucidated by analyzing the cytogenetic analysis of the retained products of conception (POC). From our understanding, we have not come across any large population-based study in India that analyzed the POC for identifying the cause of miscarriage.

Objectives: This laboratory-based research study aimed at finding the incidences of chromosomal defects from large number of POC samples received from tertiary care laboratory.

Materials and methods: The current study comprises retrospective analysis involving cytogenetic reports of large number of cases ($n = 1,732$) undertaken at the Department of Cytogenetics, Metropolis Healthcare Laboratory, Mumbai, India, between January 2014 and December 2016.

Results: Karyotypes with no abnormal findings were recorded in 82.97% cases, whereas aneuploidy was detected in 17.03% cases. Among the aneuploidy cases, they were further categorized into monosomy X (38.14%), trisomy (36.02%), double trisomy (0.85%), triploidy (15.25%), tetraploidy (8.90%), and derivatives (0.85%). Among the total study cases, 76.33% and 23.67% showed female pattern and male pattern, respectively.

Conclusion: Evaluation of POC is of immense help for the cases with history of recurrent/repeated pregnancy loss (RPL)/losses. Early detection of chromosomal aberrations will support the treating physician toward correct reasons for RPL. Also, it also helps to rule out the possibility of gonadal cell mosaicism in cases with RPL having a normal karyotype. Cases with abnormal findings are highly recommended for genetic counseling.

Keywords: Fluorescent *in situ* hybridization, Laboratory research, Products of conception, Recurrent pregnancy losses.

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INTRODUCTION

The major cause of pregnancy loss is due to fetal chromosomal aberrations. In view of this, it becomes essential to undertake laboratory investigations on the products of conception (POC). Results of such investigations will unravel the potential reasons for recurrent miscarriage.¹ Karyotyping has been the gold standard method to analyze the POC samples.^{2,3} Karyotyping helps in identification of both the structural (translocations, deletions, and inversions) and the numerical chromosomal abnormalities. One of the major drawbacks of karyotyping is that it consumes substantial diagnosis time. In comparison to other tissues, the rate of culture failure in POC is relatively higher. This is because POC tissue demands persistent maceration and fixation in formalin, which may render it to relatively higher chances of contamination. Overall, this may hamper the success of diagnosis rate of chromosomal abnormalities in POC samples.

Under this background, molecular analyzes of POC samples to identify chromosomal aberrations have been proven useful. Molecular diagnostic tests exclusively study fetal DNA, without the need for culturing and fixing tissues. Advanced molecular diagnostic tests involve probe-based identification of chromosomal aberrations. The probe-based molecular diagnostic tests include fluorescence *in situ* hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), microarray and multiplex ligation-dependent probe amplification (MLPA).⁴

The current study focused on identifying the frequency of chromosomal aberrations in POC samples. The study protocol was approved by an independent ethics committee. The study obeyed the principles of good clinical practice and the Declaration of Helsinki.

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MATERIALS AND METHODS

Type of Study

This observational and retrospective study involved the retrospective data analysis of consecutive contemporary patients who were referred to the Department of Cytogenetics, Metropolis Healthcare Laboratory, Mumbai, India, between January 2014 and December 2016. The miscarriage material from these patients was received from different regions of India, and other countries such as Dubai and Kenya. A total of 1,732 cases were considered in this study. Of the 1,732 cases, 346 showed no growth or FISH was not possible due to contamination. Therefore, a total 1,386 FISH reports, which were analyzed between the study period, were considered for this study.

Analysis of the Sample

Analysis by karyotyping and FISH of the miscarriage material was executed by trained laboratory staff per the guideline of the laboratory. The common indication for cytogenetic testing most commonly included two or more pregnancy losses and ultrasound showing multiple congenital anomalies. Proper collection and transport guidelines are provided to the center doing medical termination of pregnancy (MTP) in advance, and for Giemsa banding (GTG) studies two types of cultures for karyotyping long- and short-term cultures are setup and analyzed by GTG banding. Around 20 metaphases were studied per case, and for mosaic cases 50 metaphases were studied. If no growth in culture is observed, FISH studies were conducted and a minimum of 50 cells were studied; and in case of mosaicism, 100 cells are studied. Cytogenetic analysis of POC samples constituting fetal tissue content was undertaken by an experienced cytogenetic technician. Of the total 1,732 cases considered in this study, 346 cases showed no growth or FISH was also not possible due to contamination. Therefore, around 1,386 cytogenetic results were considered part of this study. The classification of the aberrations and karyotypes was per the guidelines of the International System for Human Cytogenetic Nomenclature 2013 (ISCN 2013). In few cases who experienced failure to grow cells while performing the cytogenetic technique, FISH molecular technique was undertaken to complete the analysis.

RESULTS

Our study included cytogenetic reports of 1,386 POC samples. Table 1 illustrates the incidences of normal and abnormal results analyzed by the karyotyping technique. Of the total 1,386 cases, 1150 (82.97%) showed apparently normal karyotype pattern or normal diploid status for 13/16/18/21/22/X and Y in FISH, while 236 (17.03%) cases showed chromosomal abnormalities. Of the 1,386 cases, 1058 (76.33%) showed female pattern and 328 (23.67%) showed male pattern. Among the aneuploidy cases, cytogenetic results revealed 36.02% trisomy, 0.85% double trisomy, 38.14% monosomy X, 15.25% triploidy, 8.90% tetraploidy, and 0.85% derivative. The most common chromosome aberration reported was monosomy X (38.14%). Trisomy 21 was detected in 14.41% cases, followed by trisomy 22 in 9.32% cases. Trisomy 18 and trisomy 16 were identified in 5.51% cases. This was followed by trisomy 13 (4.24%) in 10 cases. And, a solitary case of trisomy 20 was also identified. Of the total anomalies, triploidy and tetraploidy accounted for 15.25 and 8.90%, respectively. Out of 36 triploidy cases, XXY detected in 21 (58.33%) cases and XXX detected in

15 (41.67%) cases. Also, among the 21 tetraploidy cases, XXXX was detected in 9 (42.86%) cases and XXYY was detected in 12 (57.14%) cases. Of the 236 abnormal cases, 209 (88.56%) showed female pattern and 27 (11.44%) showed male pattern. Of the 236 cases, 2 cases (0.85%) of structural abnormalities in the form of derivative were observed. Only 1 case (0.42%) with trisomy for 13 and 21 and 1 case (0.42%) with trisomy for 15 and 21 were detected. Total number of 71 cases (5.12%) with mosaic pattern XX/XY was also observed, but this is not taken into consideration because of the possibility of maternal contamination (Fig. 1).

DISCUSSION

The POC remain an ideal tissue for elucidating recurrent pregnancy loss (RPL) by cytogenetic testing. Investigation results of chromosomal abnormalities in POC samples assist in determining the exact causes of RPL, which, in turn, help genetic counselors and physicians to support the couple about future pregnancy.⁵⁻⁷

In several instances of RPLs, cytogenetic reports of both mother and father reveal normal karyotype. Recurrent pregnancy loss in such circumstances can be due to fetal chromosomal abnormality during cell division errors or gonadal mosaicism.

Under such challenging circumstances, comprehensive assessment of POC samples becomes critical to ascertain accurate genetic reason for recurrent loss in pregnancy.

Our study reported aneuploidy in 17.03% of the POC samples. Fetal chromosomal abnormalities are the most common cause in early pregnancy loss, and they contribute the maximum for all the spontaneous abortions. Majority of the pregnancy losses with genetic as etiology show numerical abnormalities, and the most commonly seen is monosomy X, followed by triploidy, tetraploidy, and various autosomal numerical abnormalities. Chromosomal karyotype is the gold standard test for detecting numerical as well as structural chromosomal abnormalities, while FISH detects only numerical abnormalities. The POC specimen mostly shows high rates of culture failure since it suffers from maternal cell contamination. In such cases, FISH testing can be very important since FISH can be done directly on the available cells in interphase stage; while for karyotyping, we have to set up a long-term culture to make the cell divide and redivide to obtain a satisfactory numbers of cells and then to arrest the cell division to get the metaphases to study.^{8,9}

In view of these technical challenges and to improve the success rate along with making it cost-effective, our laboratory implemented a reflex testing strategy. In reflex testing strategy, if our laboratory does not obtain a karyotype/culture, then the case will be taken for FISH testing, which involves common aneuploidy detection, and reported accordingly. The reason for devising this strategy is that the repeat sampling of POC specimen cannot be achieved. Wherever applicable, with the consent of respective clinician and the concerned patient, the clinical specimen is taken for an additional FISH testing especially for microdeletion studies or the test is converted to microarray analysis.

Overall, this laboratory research study has successfully achieved the objective of finding the incidences of chromosomal defects from a large number of POC samples received from tertiary care laboratory.

CONCLUSION

Fluorescent *in situ* hybridization offers rapid diagnosis of chromosomal alterations from POC specimens in lower costs

Table 1: Frequency and pattern of chromosomal abnormality

Type of chromosomal abnormality	No. of cases (%)
Monosomy X	90
Trisomy 21	34
Trisomy 18	13
Trisomy 22	14
Trisomy 16	13
Trisomy 13	10
Trisomy 20	1
Triploidy	36
Tetraploidy	21
Double trisomy	2
Structural abnormalities	2

Fig. 1: Trisomy 16 (47,XX,+16); trisomy 20 (47,XY,+20); trisomy 18 (47,XX,+18); trisomy 21, and trisomy 15 (48,XY,+15,+21); trisomy 21 (47,XX,+21); trisomy 22 (47,XX,+22); 69,XXY; 46,XX,der(19)t(19:?) (p12,?); monosomy X; trisomy 13 and 21 FISH; trisomy21 FISH; trisomy18 FISH; trisomy XXXX,21 FISH: 4 green and 4 aqua signal indicating 4 copies of X and 4 copies of 18, respectively; Tetrasomy XXYY,18 FISH: 4 green and 4 red signal indicating 4 copies of 13 and 4 copies of 21, respectively

over karyotyping methods. In spite of advantages, FISH fails to accurately diagnose chromosomal duplications, small deletions, chromosomal translocations, and mosaicism. Therefore, our study recommended that FISH complemented with karyotyping will cover all chromosomal alterations in POC samples.

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