The Association of Clinical Characteristics with Cytogenetic Testing in Miscarriage Tissues: A Retrospective Review

Chun Feng*, Yan Yang¹, Xiao-Ling Tao¹, Xin Du¹, Jie Duan¹
¹ Gynecology Department, Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, China
*first author
# Corresponding authors: Professor Jie Duan, Gynecology Department, Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, China. E-mail: 2015103030031@whu.edu.cn

Abstract

Background It is known little about to what extent the cytogenetic abnormalities association with clinical factors including maternal age, history of miscarriage, fertilization way and ultrasonographic finding in miscarriage tissues. A comprehensive investigation had informed to reveal the relevance of the profiles of these clinical factors of miscarriage with chromosomal abnormalities and propose feasible recommendations.

Methods 478 cases of miscarriage tissue were performed by chromosomal microarray analysis between January 1, 2019, and December 31, 2019, the collected clinical data and the genetic findings were assessed using chi-squared analysis.

Results 261 cases (54.7%) were identified as chromosomal abnormalities. Trisomy took place more frequency in advanced age of pregnancy women (p < 0.05), and it was closely related to the history of miscarriage. trisomy 16 (24.1%) was predominant in the < 35 years group, whereas trisomy 15 (25.0%) was significantly more frequent in ≥35 years group. Trisomy 16, 15 and 13 were significantly more frequent in the first miscarriage, the second miscarriage and more than two times miscarriage, respectively. The positive rate in more than two times miscarriage in < 35 years group and ≥35 years group was 40.9% and 87.5%, respectively. More than two times miscarriages in < 35 years was significantly difference with ≥35 years (P=0.02).

Conclusion It is necessary to perform cytogenetic analysis to the miscarriage cases which are considered about the maternal age combined with history of miscarriage.

Keyword Miscarriage/ Advanced age/ History of miscarriage/ Chromosomal
abnormalities

**Background**

In recent years, the number of people with missed abortion has increased. Some people pay attention to the causes like environmental pollution, life style change, advanced age of pregnancy, wide application of assisted reproductive technology and so on. However, these speculations need to be confirmation. As the research further develops, people find that the gamete inherent defection or defect arising at fertilization and/or cleavage may relate to miscarriage [1]. In some studies, autosomal trisomy is the most frequent causes in the miscarriage which is indicated to advanced age of pregnancy [2-4], in other reports, it is linked to genetic abnormalities and the history of missed abortion leading to miscarriage [5-7]. Nevertheless, it is still unknown that this to what extent association with clinical factors, such as maternal age, history of miscarriage, fertilization way and ultrasonographic finding. Cytogenetic analysis of Product of conception (POC) is thought to be the most effective and efficient detection for identifying the causes of missed abortion.

Despite that the causes of missed abortion are complex, chromosomal abnormalities is one of the most important causes among these reasons. The methods for detection of chromosomal abnormalities include chromosomal karyotype analysis of POC (mainly for chorionic villous and fetal thigh muscle tissue), fluorescence in situ hybridization (FISH) and chromosomal microarray analysis (CMA). Every method has its own advantages. CMA is widely used for genetic detection, it compasses two kinds of techniques: the array comparative genomic hybridization(aCGH) and single nucleotide polymorphism (SNP) microarray techniques. At present, CMA is recommended as the optimal method for detection of apparent congenital diseases by the International Standards for Cytogenomic Arrays Consortium [8].

In this study, SNP analysis was performed to detect the cases of aborted embryonic tissues from missed abortion patients in the Abortion Ward in Maternal and Child Health of Hubei Province, Tongji Medical College of Huazhong University of Science and Technology in the whole 2019 year. A comprehensive investigation had informed
the association between chromosomal abnormalities of missed abortion and clinical characteristics, which contain maternal age, fertilization way, history of miscarriage and ultrasonographic finding. We aim at revealing the relevance of the profiles of these clinical factors of miscarriage with chromosomal abnormalities and proposing feasible recommendations.

Methods

The cytogenetic testing of 478 cases of missed abortion was managed in our inpatient ward between January 1, 2019, and December 31, 2019, the results were retrospectively reviewed. Our hospital is specialized for pregnancy women care and has a special clinic for women with pregnancy complications and related diseases. All pregnancies were clinically confirmed by the presence of an intrauterine gestational sac and the level of $\beta$-HCG in serum. Miscarriage was diagnosed by transvaginal/transabdominal ultrasound and/or combined with the level of serum $\beta$-HCG and progesterone, such as a gestational sac without fetal heart rate or a persistent anembryonic with the level of serum $\beta$-HCG which was tested at least two times was doubled unsatisfactory or a fetus without heartrate. Cases of biochemical pregnancy, ectopic pregnancy, and (vanishing) twin pregnancy were excluded from this study. Product of conception (POC) specimens were collected mostly by medical procedures, namely artificial abortion operation and induced labor. When the expulsion of POC spontaneously occurred, fetal tissue was extracted from the discharged specimens and used for testing. Maternal peripheral blood was prepared in order to eliminate the maternal interference, the tissue was placed under saline irrigation at least twice in order to remove the maternal blood component.

Data and statistical analysis

Clinical information on miscarriages was retrospectively collected from medical records. Patient age, fertilization way (natural fertilization or fertilization by assisted reproduction technology), the history of miscarriages and ultrasonographic findings were taken for investigation. In addition, cytogenetic testing indicated that two types of chromosomal abnormalities were found, the case was classified into the karyotype
grouping, which was likely to be the main cause of the miscarriage (for instance, a case with a combination of 47, XXY/XYY and trisomy was classified into trisomy as 47, XXY/XYY by itself was unlikely to be the cause, whereas, in a case with a combination of 45, X and trisomy, both abnormalities might have been the cause; thus the case was classified into the ‘mixed’ group). 46, XY, inv (9) (p12q13) was classified into the normal karyotype group because it represents a normal variant. All data were analysed using chi-squared analysis with JMP 11.2 software (SAS Institute, Cary, NC, USA); P < 0.05 was significant difference.

Results

478 women were taken cytogenetic testing of POC over the whole year in 2019 in our inpatient ward. 477 women were arranged for retrospective investigation as only one POC sample from a woman was disturbed by maternal blood. The average age of the 477 cases was 30.7 ± 2.8 years (20 – 49) and all the women were ethnically Chinese. The frequency of the karyotypes of the retained POC were illustrated in Table 1, patient age (≥ 35 years or < 35 years), fertilization way (natural fertilization or fertilization by assisted reproduction technology), number of miscarriages and ultrasonographic findings were investigated. 216 cases (45.3%) were identified as normal karyotypes and 261 cases (54.7%) were chromosomal abnormalities. These cases were included 111 (42.3%) trisomy, 33 (6.9%) monosomy, 1 (0.2%) tetrasomy, 3 (0.6%) double trisomy, 27 (5.7%) triploid or tetraploid, 53 (11.1%) mosaicism, 25 (5.2%) mixed, 1 (0.2%) uniparental disomy (UPD), and 18 (3.8%) undefined meaning chromosomal microdeletion or microduplication. With respect to investigated patients, the frequency of trisomy/ double trisomy/ euploid abnormalities/ mixed occurred more in the ≥ 35 years than in the < 35 years (P < 0.05), monosomy occurred less frequency in ≥ 35 years than in < 35 years (P < 0.05). At the same time, 48 patients were involved in this investigation fertilized by assistant reproduction technology, 25 cases (52.1%) of POC were tested chromosomal abnormalities. 25.0% was trisomy which gained the highest rate. Additionally, the number of previous miscarriages were also be observed. 214
cases (56.2%) were chromosomal abnormalities in once miscarriage, 23 cases (54.8%) in twice miscarriage and 17 cases (58.6%) in more than two times miscarriages. Trisomy occurred the most frequency in the first miscarriage, the second miscarriage and more than two times miscarriages. Trisomy happened more frequency in twice time miscarriages group (35.7%) than the other two groups (23.4% and 17.2%), chromosomal euploid abnormalities/ mosaicism/ mixed took place more frequency in more than two times miscarriages group (17.2%, 13.8%, 6.9%) than the other two groups (5.0%, 11.5%, 6.0% in the first miscarriage and 7.1%, 11.9%, 0.0% the second miscarriage), monosomy/ double trisomy/ tetrasomy/ undefined meaning CNVs was occurred more frequency in once miscarriage group than in the other two groups. On the other hand, 24 cases had abnormal fetal ultrasonographic findings, 13 cases had chromosomal abnormalities with 8 cases of trisomy and 5 cases of monosomy.

Table 2 showed the case number of trisomy (chromosome 1-22) according to age of patient and the number of previous miscarriages. With respect to patient age, trisomy 16 (24.1%) was predominant in the <35 years group, followed by trisomy 22 (20.3%). Whereas trisomy 15 (25.0%) was significantly more frequent in ≥35 years group and followed by trisomy 22 (18.8%). In regard to the number of previous miscarriages, trisomies 16, 22, 14 were significantly more frequent in the first pregnancy, trisomy 13 and 15 were significantly more frequent in once miscarriage and more than one time miscarriages, respectively (p<0.05).

Figure 1 showed the positive rate of chromosomal abnormalities regarding the maternal age and previous miscarriages. The positive rate in the first miscarriage, the second miscarriage and more than two times miscarriages in <35 years were 52.3%, 49.1% and 40.9%, respectively. The difference between the first miscarriage and the second miscarriage was not significant (P=0.66). Meanwhile, the positive rate in the first miscarriage, the second miscarriage and more than two times miscarriages in ≥35 years were 70.2%, 66.7% and 87.5%. The difference between the first miscarriage and the second miscarriage was not significant (P=0.86). More than two times miscarriages
In general population, miscarriage rate for natural conception is about 10%-15% [9]. The causes of miscarriage are complex, the underlying reasons include anatomy, endocrine abnormalities, genetics, immunization, infection, placental microcirculation disorder, environment factor and even other unknown factors. Traditional epidemiological study reveals that 50%-80% missed abortion result from genetic factor [10]. Recently some studies manifest that genetic factor account for 50-60% of all factors related to missed abortion [11]. Particularly fetal heart rate loss in the first pregnancy trimester with fetal malformation, the genetic abnormalities take up a high probability. Therefore, it is necessary to implement genetic analysis of POC to find out the probable reason, even so that POC genetic analysis sometimes show as mosaicism for placental rather than fetal tissue and it may induce diagnostic inaccuracy. Therefore, patients in our inpatient ward were suggested to take POC genetic analysis, as cytogenetic analysis cost seems high and this item is not covered by public medical insurance in China, parts of them received this analysis. The traditional detection method for chromosomal abnormalities is G-banding karyotype analysis, due to its trivial process and limitations, chromosomal microarray analysis including SNP and CGH becomes the first-tier method for the clinic detection of congenital genetic diseases and prenatal diagnosis. In this retrospective study, SNP analysis was used to detect the chromosomal abnormalities of POC (Figure 2), 1 sample was disturbed by maternal blood, the diagnostic rate was 99.8% (477/478). Chromosomal abnormalities rate was 54.7%, trisomy (23.3%) to be the most common chromosomal abnormalities, and mosaicism (11.1%) came the second. The rest of chromosomal abnormalities were monosomy, euploid abnormalities, undefined meaning chromosomal abnormalities and so on. Missed abortion with fetal malformation had almost the same rate (54.2%) of SNP analysis as without malformation. The chromosomal abnormalities were mainly for trisomy and monosomy. SNP analysis carries the limitation that it is used to certain the copy number variant but chromosomal structural abnormality, such as pericentric...
inversion and Robertsonian translocation, they could not be detected [12]. SNP used to detection for POC chromosomal abnormalities has definite boundlessness.

Maternal age is an important factor influencing pregnancy outcome. The risk of chromosomal abnormalities increases with increases in the maternal age. An advanced maternal age is the only factor that has been identified to be closely related to the risk of embryonic chromosomal abnormalities. In this paper, we also found that the rate of cytogenetic analysis was 71.6% (58/81) in the $\geq 35$ years, which was higher than in the $< 35$ years with the rate of cytogenetic analysis was 65.9%. Particularly the rate of trisomy in the $\geq 35$ years (55.2%) was significantly higher than that of the $< 35$ years (38.9%). Therefore, it is necessary for advanced age women to take a cytogenetic analysis for POC at the time of this miscarriage and move forward a better readily to next pregnancy.

Patients in this study were recommended to carry this analysis no matter how many times the miscarriage took place. We identified that the POC cytogenetic analysis had no variation according to the first or the second of miscarriage in the $\geq 35$ years group and $< 35$ years group, nevertheless, women with a history of recurrent miscarriage had a significant high possibility of cytogenetic analysis for POC in $\geq 35$ years group than that of in $< 35$ years group. Based on this result, it could be concluded that maternal age and miscarriage history should be considered whether to perform chromosomal analysis. Women with the history of less than two times miscarriage regardless of age were recommend to carry out POC cytogenetic analysis. Women with the history of recurrent miscarriage and $\geq 35$ years were requested to perform this analysis. As age grows, the probability of chromosomal mismatch increases. However, it has many other reasons but chromosomal abnormalities of multiple miscarriage for younger women. For the patients, if the causes of miscarriage are found by performing the POC cytogenetic analysis in the first time of miscarriage, they will not endure the suffering such as subsequent miscarriage and aspirin/heparin therapy and psychological burden.
Just like Foyouzi et al. indicated that carrying out a chromosomal analysis of the POC after the second pregnancy, especially among advanced age patients, provided a substantial economic advantage and it was possible to avoid unnecessary recurrent miscarriage tests [13].

Conclusions
In summary, the causes of miscarriage are complex. Cytogenetic analysis is a valuable method to ascertain the cause of miscarriage, despite its some limitations, FISH testing maybe a supplement in the future. The age of pregnancy women is an important factor affecting the POC chromosomal abnormalities. Female age combined with the history of miscarriage indicate the necessity of performing cytogenetic analysis. Although the samples from this retrospective study came from our inpatient ward through the whole 2019 year, they were not present the general population. The result is still limited, more samples are needed to confirm our conclusion. Moreover, more details about the parent genetic information are expected to collect for better reveal the causes of miscarriage.

Abbreviations
POC: Product of conception
UPD: uniparental disomy
FISH: fluorescence in situ hybridization
CMA: chromosomal microarray analysis
SNP: single nucleotide polymorphism
aCGH: array comparative genomic hybridization
CNV: copy number variant

Declarations
Ethics approval and consent to participate
The study was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology. The Affiliated Maternal and Child Health Hospital of Hubei Province provided administrative permissions for the research team to access and use the data included in this research. Data were extracted from medical records, and the consent to participate was unavailable due to the retrospective design of the study and difficulty in reconnection; however, the private information was well
protected.

**Consent for publication**
Not applicable.

**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**
The authors declare that they have no competing interests.

**Funding**
This work was supported by Hubei Health Organization Joint Project (No.WJ 2019H193). The funding sponsor had not played any role in study design, data collection, analysis, interpretation of data or writing of the manuscript.

**Authors’ contributions**
C.F. designed the study and wrote this manuscript, Y.Y. collected the primary date, X.L.T. analyzed the data, X.D. and J.D. supervised this study, and JD administered this project. All authors have read and approved the manuscript.

**Acknowledgements**
We would like to thank clinical laboratory in Maternal and Child Health Hospital of Hubei Province for their medical record offering in this study.
References

1. Page JM, Silver RM. Genetic causes of recurrent pregnancy loss. Clin Obstet Gynecol. 2016;59(3):498-508.

2. Page JM, Silver RM. Genetic causes of recurrent pregnancy loss. Clin Obstet Gynecol. 2016;59(3):498-508.

3. Hardy K, Hardy PJ, Jacobs PA, Lewallen K, Hassold TJ. Temporal changes in chromosome abnormalities in human spontaneous abortions: results of 40 years of analysis. Am J Med Genet A. 2016;170(10):2671–80.

4. Nobuaki Ozawa, Kohei Ogawa, Aiko Sasaki, et al. Maternal age, history of miscarriage, and embryonic/fetal size are associated with cytogenetic results of spontaneous early miscarriages. Journal of Assisted Reproduction and Genetics. 2019; 36:749-757.

5. Liu Y, Liu Y, Chen H, Du T, Tan J, Zhang J. The frequencies of the presence of embryonic pole and cardiac activity in early miscarriages with abnormal karyotypes. Clin Exp Obstet Gynecol. 2015;42(4):490-494.

6. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril. 2001;75(4):678-682.

7. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: a comparison of cytogenetic diagnosis in 250 cases. Obstet Gynecol Sci. 2014;57(6):518-525.

8. Miller, D.T., Adam, M.P., Aradhya, S., Biesecker, L.G., Brothman, A.R., Carter, N.P. et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am. J. Hum. Genet. 2010; 86: 749-764.

9. Nybo Andersen, A.M., Wohlfahrt, J., Christens, P., Olsen, J. and Melbye, M. Maternal age and fetal loss: population based register linkage study. BMJ. 2000; 320: 1708-1712.

10. Rubio C, Simón C, Blanco J, et al. Implications of sperm chromosome abnormalities in recurrent miscarriage. J Assist Reprod Genet. 1999;16(5):253-258.

11. Gang Li, Haixia Jin, Wenbin Niu, et al. Effect of assisted reproductive technology
on the molecular karyotype of missed abortion tissues. Biosci Rep. 2018; 38(5): BSR20180605.

12. American College of Obstetricians and Gynecologists, 2013. Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis. Obstet. Gynecol. 122, 1374-1377.

13. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: a comparison of cytogenetic diagnosis in 250 cases. Obstet Gynecol Sci. 2014;57(6):518-525.
Figure legends

Figure 1. The positive rate of chromosomal abnormalities regarding the maternal age and previous miscarriages. In <35 years group, the positive rate in the first miscarriage, the second miscarriage and more than two times miscarriage were 52.3%, 49.1% and 40.9%, respectively. In ≥35 years group, the positive rate in the first miscarriage, the second miscarriage and more than two times miscarriage were 70.2%, 66.7% and 87.5%, respectively. There were no difference in the first miscarriage and the second miscarriage between <35 years group and ≥35 years group. More than two times miscarriages in <35 years group was significantly difference with ≥35 years group. P was calculated by Chi-square test analysis; *P>0.05, **P<0.05.

Figure 2. Cytogenetic analysis of POC using SNP microarray. (A) Displays the trisomy chromosome 21 diagnostic reading obtained from miscarriage sample. (B) Demonstrates the deletion of q26→q27 reading of chromosome 6. (C) Shows the duplication of p22.3→p11.2 reading of chromosome 7. (D) Presents the trisomy chromosome 16 diagnostic reading obtained from miscarriage tissue.