J Pediat Infants 2021

Volume 4: 2

Journal of Pediatrics and Infants

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

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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.

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.

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. A survey reported that up to 75% participants strongly wished to know the causes of their miscarriage, even

Article Information

Article Type: Research
Article Number: JPI-137
Received Date: 09 February, 2021
Accepted Date: 30 June, 2021
Published Date: 05 July, 2021

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Citation: Feng C, Yang Y, Tao XL, Du X, Duan J (2021) The Association of Clinical Characteristics with Cytogenetic Testing in Miscarriage Tissues: A Retrospective Review. J Pediat Infants Vol: 4, Issu: 2 (08-13).

if there was no intervention could prevent it from occurring. Moreover, of those respondents who endured a miscarriage 41% reported feeling that they had done something wrong, 41% felt left on their own, and 28% felt ashamed [1]. Thus, identifying a potential cause of the miscarriage may influence patients' psychological and emotional responses, and possibly help in subsequent pregnancies. It is estimated that genetic factors account for 50–60% of all factors relevant to miscarriage. Particularly, for early miscarriage (gestational age <14 weeks) more than 50% of embryos have chromosomal karyotypic abnormalities. Clinically, one of the most commonly identified causes of missed abortion is chromosomal abnormality [2]. As the research further develops, people find that the gamete inherent defection or defect arising at fertilization and/or cleavage may relate to miscarriage [3]. In some studies, autosomal trisomy is the most frequent causes in the miscarriage which is indicated to advanced age of pregnancy [4-6], in other reports, it is linked to genetic abnormalities and the history of missed abortion leading to miscarriage [6-8]. 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 [9].

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. This study should help physicians working in the field to realize the clinical characteristics (maternal advanced age and history of miscarriage) contributed to chromosomal abnormalities in the cases of fetal loss. It should also be informative to the patients to understand the cause of pregnancy loss, and hopefully assist with associated grief and loss and in decision-making in regard to trying again.

Material and Methods

Samples

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 β -HCG in serum. Miscarriage was diagnosed by transvaginal/ transabdominal ultrasound and/or combined with the level of serum β-HCG and progesterone, such as a gestational sac without fetal heart rate or a persistent anembryonic with the level of serum β-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. Products 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.

Methods

All POCs were obtained from all the cases. The samples were preserved in normal saline and sent to laboratory. Under anatomic microscope, chorionic villi or fetus tissues were obtained and collected. The chorionic villi or fetus tissues were washed with phosphate buffer solution (PBS) to remove coagulated blood and decidua, and then stored at −80ºC for DNA extraction.

DNA extraction

Sample DNA was extracted with QIAamp DNAMini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was quantitated with NanoVue Plus (GE, Fairfield, Connection, U.S.A.), and then stored at −20ºC.

SNP microarray analysis

DNA (200 ng) was used as an input for a single array. DNA amplification, tagging, and hybridization were performed according to the manufacturer's protocols. The arrays were scanned on a HiScanSQ (Illumina, U.S.A.). Data analysis was performed using GenomeStudio (Illumina, standard settings). The HumanCytoSNP-12v.21 array, which covers more than 220000 markers, was employed in the present study to detect molecular karyotype, and the raw data were analyzed using GenomeStudio software (Illumina).

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 was also being 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.

	Total (n=477)	Age (years)		Fertilized by ART	Previous of miscarriages			Fetal unusual ultrasonographic findings
		<35	≥ 35		0	1	≥ 2	
Trisomy	111(23.3%)	79	32	12	89(23.4%)	15(35.7%)	5(17.2%)	8
Double trisomy	3(0.6%)	0	3	0	3(0.8%)	0(0.0%)	0(0.0%)	0
Tetrasomy	1(0.2%)	1	0	0	1(0.3%)	0(0.0%)	0(0.0%)	0
Monosomy	33(6.9%)	31	2	4	29(7.6%)	0(0.0%)	2(6.9%)	5
Triploid and tetraploid	27(5.7%)	22	5	2	19(5.0%)	3(7.1%)	5(17.2%)	0
Mosaicism	53(11.1%)	44	9	5	44(11.5%)	5(11.9%)	4(13.8%)	0
UPD	1(0.2%)	1	0	0	0(0.0%)	1(2.4%)	0(0.0%)	0
Mixed	25(5.2%)	18	7	1	23(6.0%)	0(0.0%)	2(6.9%)	0
Undefined meaning chromosomal abnormilites	18(3.8%)	15	3	1	17(4.5%)	0(0.0%)	1(3.4%)	0
Normal karyotypes	216(45.3%)	193	23	23	167	19	12	11
Abnormal karyotypes	261(54.7%)	203	58	25	214	23	17	13

Table 1: Comparison of the results of the products of conception analysis across different categories.

Trisomy	Age		Previous history of miscarriages			<35years (number of times with miscarriage)			≥ 35yeass (number of times with miscarriage)		
	<35 years	≥ 35 years	1	2	<2	1	2	<2	1	2	<2
21	11	5	14	2	0	9	1	0	4	1	0
16	19	4	20	2	1	16	2	1	4	0	0
14	3	0	2	0	1	3	0	0	0	0	0
10	2	0	2	0	0	2	0	0	0	0	0
13	9	2	6	4	0	4	4	0	2	0	0
15	2	8	6	2	2	1	0	0	5	1	2
17	1	0	1	0	0	1	0	0	0	0	0
18	4	2	4	0	1	3	1	0	1	0	1
20	1	2	3	0	0	1	0	0	2	0	0
22	16	6	19	2	0	13	2	1	6	0	0
2	1	1	2	0	0	1	0	0	1	0	0
3	2	0	2	0	0	2	0	0	0	0	0
4	1	0	1	0	0	1	0	0	0	0	0
5	1	0	1	0	0	1	0	0	0	0	0
7	3	0	3	0	0	3	0	0	0	0	0
8	3	2	3	2	0	1	2	0	2	0	0

Table 2: Trisomy outcome of the products of conception per patient age and previous history of miscarriages.

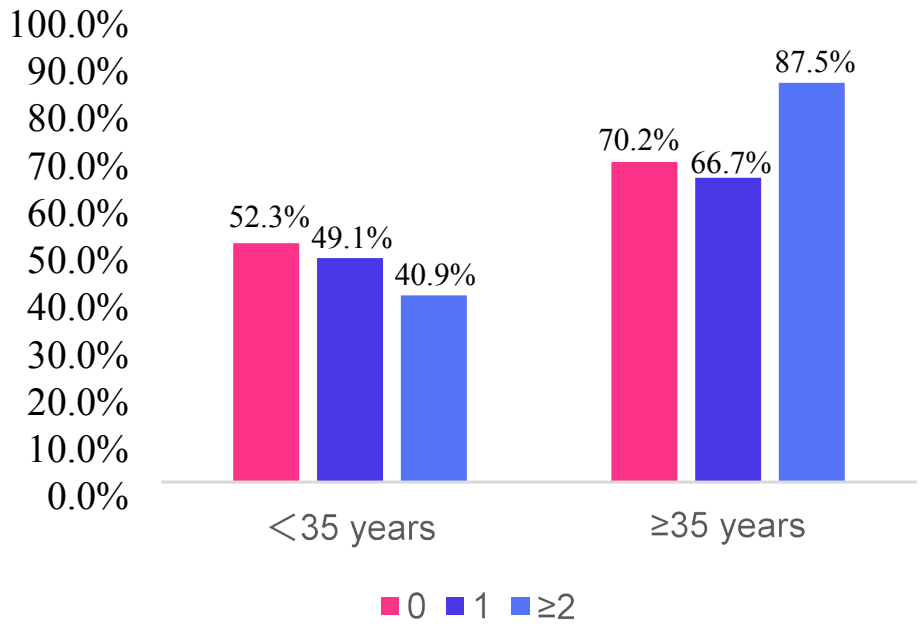

Figure 1: The positive rate of chromosomal abnormalities regarding the maternal age and previous miscarriages. In < 35 years group, the positive rate in the first miscarriage, the second miscarriage and more than two times miscarriage were 52.3%, 49.1% and 40.9%, respectively. In ≥ 35 years group, the 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.

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 <35 years was significantly difference with ≥ 35 years (P=0.02).

Discussion

In general population, miscarriage rate for natural conception is about 10%-15% [10]. The causes of miss abortion 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 [11]. Recently some studies manifest that genetic factor account for 50-60% of all factors related to missed abortion [12]. 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 [13]. 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 ≥ 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 ≥ 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 ≥ 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 ≥ 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 recommended to carry out POC cytogenetic analysis. Women with the history of recurrent miscarriage and ≥ 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

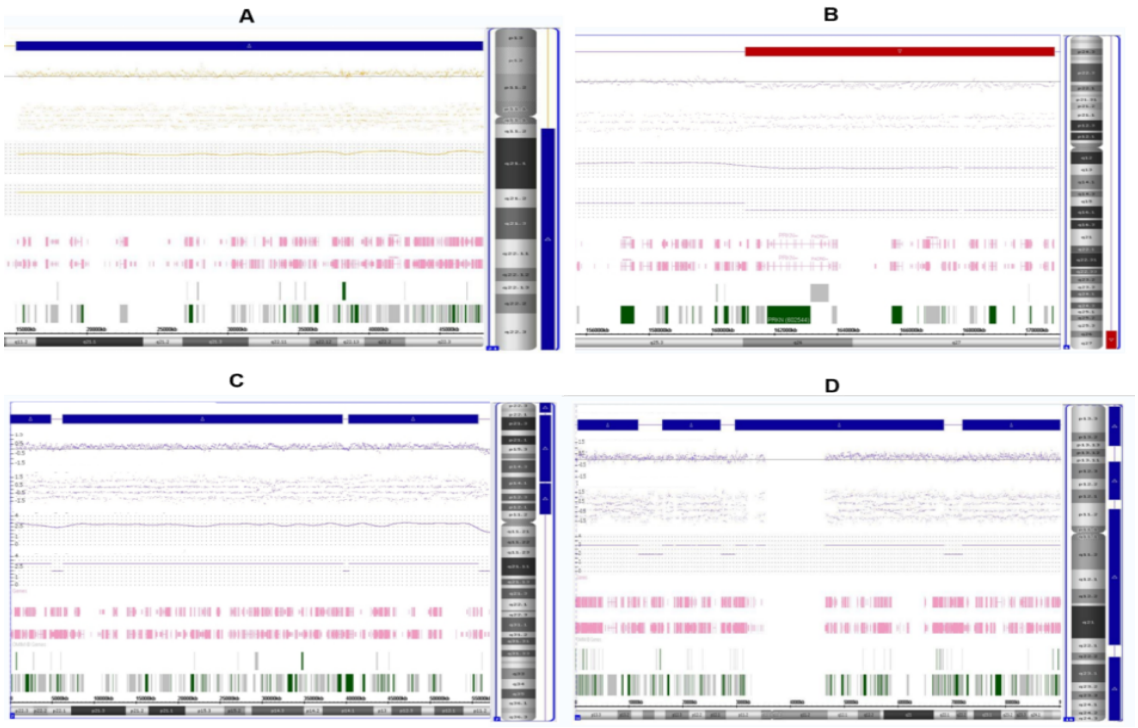

Figure 2: Cytogenetic analysis of POC using SNP microarray. (A) Displays the trisomy chromosome 21 diagnostic reading obtained from miscarriage sample. (B) Demonstrates the deletion of q26→q27 reading of chromosome 6. (C) Shows the duplication of p22.3→p11.2 reading of chromosome 7. (D) Presents the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

multiple miscarriages 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.

Conclusion

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.

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 (No.[2020]IEC(A009)). The Affiliated Maternal and Child Health Hospital of Hubei Province provided administrative permissions for the research team to access and use the fetal tissue for testing and clinic 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 interest

The authors declare that they have no competing interests.

Funding

This work was supported by National Youth Foundation of China (No. 82001644). The funding sponsor had not played any role in study design, data collection, and analysis, interpretation of data or writing of the manuscript.

Authors' contribution

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. All authors have read and approved the manuscript.

Acknowledgement

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

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Citation: Feng C, Yang Y, Tao XL, Du X, Duan J (2021) The Association of Clinical Characteristics with Cytogenetic Testing in Miscarriage Tissues: A Retrospective Review. J Pediat Infants Vol: 4, Issu: 2 (08-13).