Correlation of Maternal Age and Chromosomal Abnormality in Products of Conception- A Single Centric Study

By Dr. Yamini Jadhav, Vidya Bhairi, Ajinkya Jadhav, Bhagyashree Gedam, Vedant Patole, Shweta Parab & Dr. Jayaram Kandandale

Abstract- Purpose: The purpose of this study is to find out the types and incidence rate of chromosomal abnormalities and the relationship between maternal age and chromosomal abnormality in products of conception by retrospective analysis.

Method: Karyotype study using standard GTG banding and FISH study for aneuploidy detection was done from products of conception samples.

Results: A total of 513 cases of products of conception were studied retrospectively. 98 cases were studied by conventional cytogenetic technique and 415 cases were studied by a FISH method. The chromosomal abnormality was observed in 97 cases (18.91%). Trisomy was found to be a major chromosomal abnormality amongst the cases studied, followed by triploidy and monosomy. A higher incidence of chromosomal abnormality was found in women with advanced maternal age as compared to the maternal age less than 35 years.

Keywords: karyotype, products of conception, FISH, chromosome abnormality, maternal age.

GJMR-E Classification: NLMC Code: QS 677, WP 400
Correlation of Maternal Age and Chromosomal Abnormality in Products of Conception-A Single Centric Study

Dr. Yamini Jadhav¹, Vidya Bhairi¹, Ajinkya Jadhav¹, Bhagyashree Gedam², Vedant Patole³, Shweta Parab⁴ & Dr. Jayaram Kandandale⁵

Abstract: Purpose: The purpose of this study is to find out the types and incidence rate of chromosomal abnormalities and the relationship between maternal age and chromosomal abnormality in products of conception by retrospective analysis.

Method: Karyotype study using standard GTG banding and FISH study for aneuploidy detection was done from products of conception samples.

Results: A total of 513 cases of products of conception were studied retrospectively. 98 cases were studied by conventional cytogenetic technique and 415 cases were studied by a FISH method. The chromosomal abnormality was observed in 97 cases (18.91%). Trisomy was found to be a major chromosomal abnormality amongst the cases studied, followed by triploidy and monosomy. A higher incidence of chromosomal abnormality was found in women with advanced maternal age as compared to the maternal age less than 35 years.

Conclusions: Pregnancy loss is high in women with advanced maternal age group. The study of products of conception helps the couple to recognize the cause of miscarriage. These results help the couple to cope up with the emotional burden of miscarriage and in the future pregnancy management.

Keywords: karyotype, products of conception, FISH, chromosome abnormality, maternal age.

I. Introduction

Advanced maternal age is defined as a pregnancy in women at 35 years of age or more regardless of parity, whether the conception is first or not. Reproductive health is a state of complete mental, physical, and social well-being related to all stages of reproductive processes [1]. Delayed motherhood due to carrier opportunities and changes in marriage pattern, use of contraception, social support, and other possible factors such as stress, pollutants, and smoking habits are responsible for the increase in the rate of pregnancy loss or miscarriage. The trend of delaying pregnancy has been observed worldwide.

Corresponding Author: Clinical Cytogenetics Department, Lilac Insights Pvt. Ltd., Navi Mumbai, (Maharashtra), India.
e-mail: yamini.jadhav@lilacinsights.com
Author ¹: Clinical Cytogenetics Department, Lilac Insights Pvt. Ltd., Navi Mumbai, (Maharashtra), India.
Author ²: Clinical Cytogenetics Department, Lilac Insights Pvt. Ltd., Navi Mumbai, (Maharashtra), India.
Author ³: Department of Clinical and Molecular Cytogenetics, Centre for Human Genetics, Bangalore, (Karnataka), India.

II. Material and Methods

a) Study Specimens

The retrospective study was performed on the products of conception samples received at the clinical cytogenetics department. Proper collection and transport guidelines for POC sample collection are circulated and explained to the centers sending the samples. For all first-trimester pregnancy losses, the abortus material was collected in a sterile container with transport media under aseptic precautions.

The results were archived from the laboratory database, and in addition to maternal age, no personal information from the patients was included. As the laboratory receives material from different medical facilities with limited information, clinical data such as gestational age at the time of abortion and clinical
history of the parents were not available for all the samples received. A total of 513 results from POC analysis were performed.

Our study has been performed in two groups. Group I consists of cases with maternal age 18 to 34 years, and Group II consists of cases with advanced maternal age group (Age 35 years and above).

b) Cytogenetic and FISH analysis

All the POC specimens received were cleaned to remove decidual tissues as well as bloodstains. Then the tissue samples were digested using trypsin and collagenase. After tissue digestion, it was divided for the culture set up for karyotype and the FISH study. The karyotype, as well as FISH, was done using the standard protocol. The FISH study was performed for aneuploidy detection which includes chromosomes 13, 18, 21 and sex chromosomes. For each probe mix, 50 interphase cells were studied.

The GTG banding was done for the karyotype study, and for each case a minimum of 20 metaphases was analyzed. Karyotype nomenclature was designated as per an international system for human cytogenomic nomenclature (ISCN 2016) [8].

III. Results

Out of 513 cases 416 cases showed normal results for Group I and II and 97 cases showed chromosomal abnormalities (Table 1). The rate of abnormality in the overall study was 18.91% (97/513).

Table 1: Distribution of studied cases

| Age Group          | n   | 18-34 Years | 35 Years and above |
|--------------------|-----|-------------|--------------------|
| NORMAL             | 416 | 361 (83%)   | 55 (71%)           |
| ABNORMAL           | 97  | 75 (17%)    | 22 (29%)           |
| TOTAL              | 513 | 436         | 77                 |

Out of the 97 abnormal cases trisomy was seen in 45.36% (n=44) cases followed by monosomy and triploidy in 25.77% (n=25) and 27.84% (n=27) each.

Monosomy X was seen in 23.71% of cases followed by Trisomy 18 in 18.56%, Trisomy 21 in 13.4% and Trisomy 13 in 9.28% cases (Table 2).

Table 2: Distribution of chromosomal abnormality based on age group

| Chromosome anomaly | Age Group             | Total Abnormal Cases (%) |
|--------------------|-----------------------|--------------------------|
|                    | 18-34 Years | 35 Years and above |          |
| Trisomy 6          | 1           | 0                       | 1 (1.03%)  |
| Trisomy 7          | 0           | 1                       | 1 (1.03%)  |
| Trisomy 10         | 0           | 1                       | 1 (1.03%)  |
| Trisomy 13*        | 7           | 2                       | 9 (9.28%)  |
| Trisomy 16         | 1           | 0                       | 1 (1.03%)  |
| Trisomy 18         | 12          | 6                       | 18 (18.56%)|
| Trisomy 21**       | 8           | 5                       | 13 (13.40%)|
| Monosomy X         | 20          | 3                       | 23 (23.71%)|
| Monosomy 18        | 1           | 0                       | 1 (1.03%)  |
| Monosomy 21        | 0           | 1                       | 1 (1.03%)  |
| Triploidy          | 24          | 3                       | 27 (27.84%)|
| Tetraploidy        | 1           | 0                       | 1 (1.03%)  |
| **46, der(14;21)(q10;q10), +21 was counted in Trisomy 21 group.**

In terms of abnormality, we found that, the highest frequency was Trisomy followed by Monosomy X and Trisomy 18.

In the age group below 35 years, 83% had normal results, whereas abnormalities were found in 17% of cases. For the advanced maternal age group (Age 35 years and above), normal results were found in 71% of cases and abnormal results in 29% of cases. The rates of abnormal results were significantly higher for the advanced maternal age group when compared to the younger maternal age group (Figure 1).
IV. Discussion

The etiology of pregnancy loss is heterogeneous which involves association among maternal, paternal and placental or fetal risk factors in pathways associated with conception and fetal growth. The maternal risk factor includes infection, endocrine, anatomic, immunological and genetic abnormalities whereas embryonic defects such as chromosomal abnormality reduces embryonic development. The rate of miscarriage is higher after the age of 30-35 years due to declined potential fertility.

For couples with a history of recurrent miscarriages, it is ideal to study the POC specimen where couples karyotype study shows normal karyotype results. Hence to know the genetic etiology behind the pregnancy loss and it is recommended to study POC specimen, where fetal chromosomal abnormality can be ruled out (if any).

Different types of chromosomal abnormalities are linked with different clinical states. The occurrence of trisomy increases with increasing maternal age, which is due to the meiotic non-disjunction that occurred during gametogenesis. In the present study, the most frequent trisomy type is trisomy 18, followed by trisomy 21 and trisomy 13. Aneuploidies account for the largest amongst the abnormalities detected in POC, same as Menasha et al. [3].

The most commonly observed chromosomal abnormality is Trisomy followed by triploidy which is resulted due to abnormal fertilization. The presence of autosomal monosomy is very rare in pregnancy loss. In the present study we have found one case with monosomy 21 and monosomy 18 each. Monosomy X is most frequently observed amongst pregnancy losses. Twenty-three cases of monosomy X were found in our study. The structural abnormalities found in pregnancy loss are mainly translocations and inversions. In our study, the structural abnormalities were observed in 3 cases along with trisomy. One case of trisomy 21 along with translocation involving chromosome 14 and 21, karyotype result: 46, der(14;21)(q10;q10),+21. One case of trisomy 13 along with translocation involving chromosome 13 and 15, karyotype result: 46, der(15)t(13;15)(q10;q10),+13 and one case of trisomy 13 along with double translocation, karyotype result: 46, t(8;12), rob(13;14),+13 was seen in the study.

Presence of chromosomal abnormality in one of the parents results in a structural abnormality in the fetus. About 2-5% of couples with translocations experience repeated pregnancy losses [9-12].

The frequency of translocation amongst the couples with recurrent pregnancy losses is 40% for Robertsonian translocation and 60% for reciprocal translocations [13]. As compared to male partners, the balance chromosomal abnormalities are found twice in female partners. This is due to the fact that, the chromosomal abnormalities that are compatible with female fertility may result in male sterility.

In our study we have found higher abnormality rate (29%) in advanced maternal age group as compare to younger population studied (17%). The present findings consistent with the studies that reported the increased incidence of chromosomal abnormality with increasing maternal age [14-16]. Various studies have concluded that the women’s with advanced maternal age have a higher incidence of unfavourable reproductive outcomes such as complications during pregnancy, spontaneous pregnancy loss, infertility or congenital anomalies in foetus as compared to the younger women which is consistent with the present study [17-22].
Study of products of conception using karyotype technique is highly suggested as it studies structural as well as numerical abnormalities. It has some drawback such as longer time, for reporting, laborious processing, and culture failure. Since we receive products of conception samples from various locations for testing, the FISH method plays an important role for aneuploidy detection of chromosome 13, 18, 21, X and Y. Advances in new techniques, help to improve the detection of abnormalities and subsequently increases the diagnostic abilities. The use of interphase FISH has allowed cytogenetic setups to study POC samples that have failed to grow hence failed to report the results.

V. Conclusion

Pregnancy loss is high in women with advanced maternal age group and should be taken into consideration during pregnancy planning. Irrespective of previous pregnancy outcomes, maternal age at the time of conception is an independent and strong risk factor for fetal demise.

For the cost-effective management of the couple with a history of recurrent pregnancy losses, genetic evaluation for chromosomal abnormalities, if any, in POC samples plays an important role in avoiding the expensive non-genetic work up and also to understand the etiology behind pregnancy losses.

Genetic counseling is important in the clinical management of pregnancy losses to identify the recurrent risk in future pregnancy influenced by karyotype results. Hence finding this information in an accurate, fast and, reliable manner is critical. Other molecular testing methods such as array CGH and sequencing are useful in the detection of numerical aberrations such as monosomy or trisomy. Structural chromosomal aberrations, tetraploidy, polymorphism may be difficult to identify depending on the method used for detection. Hence the possible algorithm for assessing products of conception could be conventional karyotype study followed by FISH testing, which can identify the common trisomy, tetrasomy, or polyploidy.

New technologies such as Microarray, NGS studies of POC are still not commonly used due to high cost, the difficulty of CNV interpretation, inability to detect balanced chromosomal translocation, and limitation of ploidy change detection in some microarray platforms. However, both these techniques enable fast genetic testing and atomization; we believe that these testing will be implemented in wide practice soon.

The limitation of present study is small sample size and unavailability of maternal cell contamination data hence, further study is necessary to address these problems.

Acknowledgements

The authors acknowledge the help and support of Lilac Insights Pvt. Ltd., Navi Mumbai, India for the study.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References Références Referencias

1. WHO guidlines WHO: Reproductive health. Available from: https://www.who.int/westernpacific/health-topics/reproductive-health
2. Wang BT, Chong TP, Boyar FZ, et al. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. Mol Cytogenet. 2014; 7: 33.
3. Menasha J, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med. 2005; 7(4): 251-263.
4. Pylyp LY, Spynenko LO, Verholyad NV, et al. Chromosomal abnormalities in products of conception of first-trimester miscarriages detected by conventional cytogenetic analysis: a review of 1000 cases. J Assist Reprod Genet. 2018; 35(2): 265-271.
5. Imam SN, Shamsi MB, Kumar K, et al. Idiopathic recurrent pregnancy loss: role of paternal factors; a pilot study. J Reprod Infertil. 2011; 12(4): 267-276.
6. Lathi RB, Gray Hazard FK, Heerema-McKenney A, et al. First trimester miscarriage evaluation. Semin Reprod Med. 2011; 29(6): 463-469.
7. Liu S, Song L, Cram DS, et al. Traditional karyotyping vs copy number variation sequencing for detection of chromosomal abnormalities associated with spontaneous miscarriage. Ultrasound Obstet Gynecol. 2015; 46(4): 472-477.
8. McGowan-Jordan, J, Simons A, & Schmid M. (Eds.) 2016 ISCN (2016): An international system for human cytogenomic nomenclature. Basel: S. Karger
9. De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod. 1990; 5(5): 519-528.
10. Pokale Y and Khadke P. Evaluation and Contribution of Major Chromosomal Abnormalities in Couples with Recurrent Miscarriage. IOSR-JDMS.2016; 15(1): 78-83.
11. Priya PK, Mishra VV, Roy P, Patel H. A Study on Balanced Chromosomal Translocations in Couples with Recurrent Pregnancy Loss. J Hum Reprod Sci. 2018; 11(4): 337-342.
12. Sheth FJ, Liehr T, Kumari P, et al. Chromosomal abnormalities in couples with repeated fetal loss: An
Indian retrospective study. Indian J Hum Genet. 2013; 19(4): 415-422.

13. Zhang T, Sun Y, Chen Z, Li T. Traditional and molecular chromosomal abnormality analysis of products of conception in spontaneous and recurrent miscarriage. BJOG. 2018; 125(4):414-420.

14. Choi TY, Lee HM, Park WK, et al. Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. Obstet Gynecol Sci. 2014; 57(6): 518-525.

15. Horiuchi I, Wakimoto Y, Kuwata T, et al. Cytogenetic Analysis of Spontaneous Miscarriages Using Long-Term Culturing of Chorionic Villi. J Fetal Med. 2019; 6:1–6.

16. Jia CW, Wang L, Lan YL, et al. Aneuploidy in Early Miscarriage and its Related Factors. Chin Med J (Engl). 2015; 128(20): 2772-2776.

17. Fretts RC, Schmittdiel J, McLean FH, et al. Increased maternal age and the risk of fetal death. N Engl J Med. 1995; 333(15): 953-957.

18. Spandorfer SD, Avrech OM, Colombero LT, et al. Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. Hum Reprod. 1998; 13(2): 334-338.

19. Nybo Andersen AM, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based register linkage study. BMJ. 2000; 320(7251): 1708-1712.

20. De la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod. 2002; 17(6): 1649-1656.

21. Homan GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. Hum Reprod Update. 2007; 13(3): 209-223.

22. Garrisi JG, Colls P, Ferry KM, et al. Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil Steril. 2009; 92(1): 288-295.