DOI: 10.24938/kutfd.1257034 Kırıkkale Üniversitesi Tıp Fakültesi Dergisi 2023;25(2):193-199
Original Article *Özgün Araştırma*

CHALLENGES FOR CHROMOSOME ANALYSIS IN PREGNANCY LOSS: LESSONS LEARNED FROM 1208 SAMPLE EXPERIENCE

Gebelik Kaybında Kromozom Analizinin Zorlukları: 1208 Örnek Deneyiminden Çıkarılan Dersler

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ABSTRACT

Objective: Previous studies have shown that half of the spontaneous abortions were associated with fetal chromosomal abnormalities, however it is not always possible to reveal the reasons of pregnancy loss. The aim of the current study was to investigate the frequency of chromosome abnormalities and culture failure rates of pregnancy loss and compare the results with similar studies in the literature.

Material and Methods: The karyotype analysis results of 1208 abortion materials which were obtained from long-term cultures of chorionic villus samples of the patients who were admitted to the Haseki Training and Research Hospital Genetic Diagnosis Center between August 2016 and February 2021, were evaluated retrospectively.

Results: No results were obtained due to culture failure in approximately half of the materials. Numerical anomalies were observed in 87% (116) of abnormal karyotypes that consist of trisomy, monosomy X and triploidy. Trisomies were the most common anomaly. While the frequency of trisomy was significantly higher in the older age group (≥35 years) (p=0.001), the frequency of monosomy X and triploidy were higher in the younger age group. Chromosomal changes whose frequencies were not affected by maternal age were structural chromosomal abnormalities and tetraploidies.

Conclusion: Since the developing new Technologies are still not affordable enough and their widespread use is limited. As a result, current approaches have indicated that chromosome analysis is still a necessary and useful method. It is thought that detecting the chromosomal anomaly that led to abortion facilitates multidisciplinary patient management and enables to provide more accurate and comprehensive genetic counseling. In cases where the chromosome analysis test is not informative, the application of DNA-based tests such as Quantitative Fluorescence PCR (QF-PCR) and molecular karyotyping may help the diagnosis.

Keywords: *Karyotyping, habitual abortion, spontaneous abortion, maternal age, chromosomal aberrations*

ÖZ

Amaç: Önceki çalışmalar spontan düşüklerin yarısının fetal kromozomal anormallikler ile ilişkili olduğunu göstermiştir, ancak gebelik kayıplarının nedenlerini ortaya koymak her zaman mümkün değildir. Bu çalışmanın amacı, gebelik kayıplarında kromozom anormalliklerinin sıklığını ve kültür başarısızlık oranlarını araştırmak ve elde edilen sonuçları, literatürdeki benzer çalışmaları karşılaştırmaktır.

Gereç ve Yöntemler: Haseki Eğitim ve Araştırma Hastanesi Genetik Tanı Merkezi'ne Ağustos 2016-Şubat 2021 tarihleri arasında kabul edilen, abortus materyali koryon villus örneklerinin uzun süreli kültürlerinden elde edilen 1208 adet düşük materyalinin karyotip analiz sonuçları retrospektif olarak değerlendirildi.

Bulgular: Materyallerin yaklaşık yarısında kültür başarısızlığı nedeniyle sonuç elde edilemedi. Trizomi, monozomi X ve triploididen oluşan anormal karyotiplerin %87'sinde (116) sayısal anomaliler gözlendi. Trizomiler en sık görülen anomali iken, ileri yaş grubunda (≥35 yaş) trizomi sıklığı anlamlı olarak daha yüksek, genç yaş grubunda monozomi X ve triploidi sıklığı daha yüksekti. Sıklıkları anne yaşından etkilenmeyen kromozomal değişiklikler, yapısal kromozomal anormallikler ve tetraploidiler idi.

Sonuç: Gelişen yeni teknolojilerin henüz yeterince ucuz olmaması ve yaygın kullanımlarının sınırlı olması nedeniyle güncel yaklaşımlar, kromozom analizinin hala gerekli ve yararlı bir yöntem olduğunu ortaya koymuştur. Düşüklere neden olan kromozomal anomalilerin saptanmasının multidisipliner hasta yönetimini kolaylaştıracağı aşikardır. Kromozom analizi testinin bilgi verici olmadığı durumda ise kantitatif floresan PCR (QF-PCR), moleküler karyotipleme gibi DNA temelli testlerin uygulanması tanıya yardımcı olabilmektedir.

Anahtar Kelimeler: *Karyotipleme, habituel abortus, spontan abortus, anne yaşı, kromozom anomalisi.*

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Received / Geliş Tarihi: 27.02.2023 **Accepted** / Kabul Tarihi: 21.06.2023

KÜ Tıp Fak Derg 2023;25(2):193-199
Doi: 10.24938/kutfd.1257034

INTRODUCTION

Pregnancy loss is one of the most common health problems seen in one out of every four women with a history of pregnancy (1). The loss of the embryo in the first trimester is called early pregnancy loss with a rate of 15% in clinically defined pregnancies (2). Furthermore, the loss of two or more pregnancies before the 20th week is accepted as recurrent pregnancy loss. In these patients, karyotyping of pregnancy tissue for explanatory purposes is generally recommended (3-4).

It was displayed that about half of pregnancy losses were due to chromosomal abnormalities (5). The rate of chromosomal anomaly detection in spontaneous abortion materials, which were examined in large series previously, ranged from 24% to 65% (6-14). In all series; more than 90% of abnormal results consisted of numerical chromosomal abnormalities such as trisomies, polyploids and monosomy X. The most common trisomy was Trisomy 16 which was followed by the trisomies of chromosomes 13, 18, 21 and 22, respectively. While nearly half of the structural chromosomal abnormalities were de novo, the other half were secondary to the familial balanced translocations and inversions in the parents.

To the best of our knowledge, the current study included the largest case series published in Turkey. The main purpose of this study is to assess the results of chromosomal analysis of 1208 abortion materials admitted to our center in a time period of approximately 5 years. According this assessment, we also aimed to determine the frequencies of chromosomal anomalies and to evaluate the relationship between the maternal age and the determined chromosomal anomalies.

MATERIALS AND METHODS

The study protocol was approved by the Clinical Research Ethics Committee of the Haseki Training and Research Hospital (decision no: 2021/89, date: 06.10.2021). The results of the conventional cytogenetic analysis of 1208 spontaneous abortion materials admitted to the Haseki Training and Research Hospital Genetic Diagnosis Center between August 2016 and February 2021 were analyzed cross-sectionally. Between 18-49 years old female patients whose spontaneous abortion materials were accepted were included in the study. Patients whose long-term culture could not be assessed appropriately, were excluded from the present study.

The samples that were brought in falcon tubes containing transport medium with informed consent forms were accepted by our laboratory, after that the samples underwent the stages of sowing, culturing, harvesting, spreading, staining and analyzing, respectively. In the sowing process; firstly, the abortion material was taken into a petri dish, in the next step the chorionic villus of the sample was separated to be divided into small pieces with a scalpel, and then 3 ml of trypsin was added and the mixture was incubated at 37ºC for 3 hours. Subsequently, it was divided into three separate culture vessels, after adding 3 ml of medium (BIOAMF-2 Medium-Biological Industries) these vessels were incubated at 37ºC in a CO^2 oven. On the 7th day of sowing, adherence levels and colony numbers were checked under an inverted microscope (Olympus CKX31, Olympus, Southall, UK) and addition of new medium was performed which was defined as feeding. The decision of harvesting was made when the cell proliferation was at least 6-8 colonies and reached the stage of division. Before the harvest, 100 microliters of colcemid (colcemid solution, Gibco) was added and incubation was continued for 2.5 hours. After the cells were split by adding 1-1.5 ml of trypsin, the contents of the flasks were transferred to the tube and centrifuged at 1200 rpm for 10 minutes. After removing the supernatant, a hypotonic solution was applied for 15-30 minutes according to the ambient conditions and it was washed 3 times with a fixative. Then, the material which was spread on slides, was left for aging process at 80 ºC for overnight. At the end of this period, the dyeing process was started with trypsin and Leishmann dye to visualize the cytogenetic bands and examine them under the microscope. Twenty metaphases, at least 5 of which were karyotyped with the Argenit Imaging system (Argenit, İstanbul, Türkiye), were analyzed in each

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patient. Results were reported according to the International System for Human Cytogenetic Nomenculature (ISCN) 2016.

Statistical Analyses

Statistical analyzes were performed using SPSS v.21. The data were presented as percentages.

RESULTS

In the present study, 600 abortion materials with successful long-term culture results were examined, and it was found that while 76% (454) of these results were normal, 24% (146) were abnormal. Of the samples with normal results, 79% (n=469) were reported as 46,XX and 21% as 46,XY, the 46,XY/46,XX gender ratio was 0.26 (97/372).

Numerical anomalies were observed in 87% (n=131) of abnormal karyotypes that consist of trisomy, monosomy X and triploidy with the rates of 50% (66), 19% (26) and 16% (21), respectively. Tetraploidy was found in 3 cases and autosomal monosomy in 1 case. Structural anomalies were detected in 10 samples (7%), most of which were translocation and inversion. In 5 cases, both numerical and structural chromosomal anomalies were observed (Table 1).

After grouping the examination material according to maternal age (Table 2); It was determined that 45% (59) of them were aged 35 and over, and 55% (72) were under the age of 35. Trisomies were the most common anomaly in both groups. While the frequency of trisomy was significantly higher in the older age group (≥35 years) with a rate of 70% of all anomalies, the frequency of monosomy X and triploidy were higher in the younger age group (<35 years).

Table 1: Chromosome analysis results in miscarriage samples

Chromosomal abnormality	Frequency (n=131)	Percent
Numerical chromosomal abnormalities	115	87.7
Monosomy X	26	19.8
Triploidy	21	16.0
Trisomy 21	12	9.2
Trisomy 16	10	7.6
Trisomy 22	7	5.3
Trisomy 18	4	3.1
Trisomy 15	4	3.1
Trisomy 14	4	3.1
Trisomy 4	2	1.5
Trisomy 2	2	1.5
Trisomy 9	3	2.3
Trisomy 10	3	2.3
Trisomy 13	3	2.3
Tetraploidy	3	2.3
92,XXXX,der(9)(9pter→9q32::?)	1	0.8
Triploidy, Trisomy15, Trisomy16	1	0.8
Trisomy 12	1	0.8
Trisomy 16, Trisomy 21	1	0.8
Trisomy 20	1	0.8
Trisomy 3	1	0.8
Trisomy 3, Trisomy 21	1	0.8
Trisomy 5	1	0.8
Trisomy 6	1	0.8
Trisomy 7	1	0.8
Trisomy 8	1	0.8
Structural chromosomal abnormalities	16	12.2
45,X,t(1;16)(q24;q12)	1	0.8
46,XY,der(15)	1	0.8
46,XY,del(5)(p14)	1	0.8
46,XX,der(15)t(7;15)(q11.2;q24)	1	0.8
46,XX,der(18)t(3;18)(q23;q21.1)	1	0.8
46,XX,der(7)t(3;7)(q21;q32)	1	0.8
46,XX,inv(12)(p11.2q13)	1	0.8
46,XX,rob(14;21)(q10;q10),+14	1	0.8
46,XY,rob(13;14)(q10;q10),+13	1	0.8
46,XY,rob(13;14)(q10;q10),+14	1	0.8
45,XX,rob(14;21)(q10;q10)	1	0.8
47,XX,t(2;4)(p25.3;p15.1)pat,+15	1	0.8
46,XX,t(2;10)(q21;p11.2)	1	0.8
46,XX,t(1;14)(q25;q24)	1	0.8
47,XY,t(2;9)(q22-24;q21),+9	1	0.8
46,XX,t(3;8)(p13;p23)	1	0.8

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Table 2: The correlation between karyotype results and maternal age

	≥35 years n=59	**<35 years n=72**
Abnormalities	Frequency % (n)	Frequency % (n)
Trisomy	71.2 (42)	33.3 (24)
Monosomy X	10.2 (6)	27.8 (20)
Structural	6.8 (4)	8.3 (6)
Mix	5.1 (3)	2.8 (2)
Triploidy	5.1 (3)	25 (18)
Tetraploidy	1.7 (1)	2.8 (2)

DISCUSSION

Most pregnancies associated with chromosomally abnormal fetuses result in abortion. The detection rate of chromosomal anomaly in large series varies between 24-65% (6-14). In the current study, this rate, which is 22%, is at the lowest level according to the literature, furthermore it was reported as 24% by Okten G et al. from Türkiye (14). In our opinion, the young maternal age, the limited number of patients and also the high rate of maternal contamination in the population of the study of Okten G. et al were the possible reasons of the difference from the other studies (6-14).

Additionally, performing chromosome analysis particularly in recurrent miscarriages might be another reason of the low rates that were observed in Türkiye. In Ogasawara's series, the rate of chromosomal anomaly detection in recurrent abortions was found to be lower than in spontaneous abortions (15). It has been suggested that this result, which was also supported by large series reported previously, is due to the role of extrachromosomal causes in recurrent miscarriages (12). It is thought that the majority of recurrent abortions in the group we examined may be one of the reasons explaining the low rate of chromosomal anomaly detection.

The major problem in cytogenetic analysis of abortion material is the maternal cells that cannot be distinguished from fetal tissue and lead to contamination. In some cases, the presence of maternal contamination can be detected by performing additional molecular tests. However when this is not possible, 46,XX normal karyotype can be reported in fetuses with chromosomal anomalies. The most common consequences of this situation are the low sex ratio of 46,XY/46,XX and low ratio of fetuses with abnormal karyotypes.

According to the results obtained in our center, the gender ratio of 46,XY/46,XX was 0.26 which might indicate maternal contamination. There was no clear limit for this rate in the literature, and it was ranging from 0.66 to 2.79 in different studies. It was thought that the difference in rates is due to the different protocols followed by the centers during chromosome analysis (11). The low rate which we presented means that possibly we were not able to detect chromosomal anomalies in some fetuses due to maternal cell contamination, therefore we might report actual chromosomal abnormalities as normal.

The common feature of the centers that could accomplish to minimize maternal contamination was possibly applying an aggressive protocol during the chromosome analysis. The protocol that they preferred, was based on counting all cells in the preparation for detecting a possible non-46,XX cell, and editing the report in this direction if Y chromosome or abnormal chromosome establishment was found in two separate culture dishes. In any debate, the FISH technique is used (11). In the implementation of this protocol, a high-tech device infrastructure and well experienced personnel are needed, furthermore it is necessary to spend enough time on each patient. In a center (Cyto Labs, Perth, Australia), with previous sex distribution rate of 1.60, reported that they accomplished to increase the rate to 2.79 after a protocol change (11). Exclusion of fetuses with 46,XX chromosome establishment is another option to minimize the effects of maternal contamination on the rate of chromosomal anomaly detection. When we reviewed our results after excluding fetuses with 46,XX, the frequency of chromosomal anomaly that was obtained, was 57%, which was comparable with the literature.

Although the chromosomal anomaly distribution observed in our study was similar to the frequencies that

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were shown in previous studies, there are still some slight differences in the frequencies and type of the anomalies. Trisomy of all chromosomes was detected except chromosomes 1, 11, 17 and 19. When classified according to the chromosome size, in accordance with the literature; while trisomies of large chromosomes (pairs 1-5) and small chromosomes (pairs 19-22) accounted for 9% and 30% of all trisomies respectively, the trisomies of medium chromosomes (pairs 6-18) was 60% (9). Although trisomy 16 is generally known as the most common trisomy, in our study we observed that trisomy 21 was the most common with a rate of 18% of all trisomies (other studies: 8-15%). In our study, trisomy 16 was detected in 15% of all as the second most common trisomy, followed by trisomy 22, which constituted 10% of all (9,16,17). Double trisomy, which was an extremely rare abnormality, was seen in 3 cases in the current study. Additional triploidy was also determined in one of these patients.

The frequency of monosomy was 20% which was compatible with previous reports (9,18,19). Monosomy was the second most common anomaly in the younger age group and was also usually seen in the same population.

It is known that the frequency of some chromosomal diseases such as aneuploidies is strongly related to the age of the mother. When we grouped our cases according to the mother age, the most common chromosomal anomaly in both groups was trisomies ($p<0.001$) (Table 2). While the frequency of trisomy was obviously higher in the older group, as expected, the frequencies of triploidy and monosomy X were higher in the younger group, with a statistically significant difference ($p<0.001$). Chromosomal changes whose frequencies were not affected by maternal age were structural chromosomal abnormalities and tetraploidies. Current approaches have revealed that chromosome analysis is still a necessary and useful method, since the developing new technologies are still not affordable enough and their widespread use is limited. Conventional cytogenetic analyzes help to determine the etiology in approximately half of the cases with recurrent pregnancy loss, which is one of the leading health problems of the population. However, with new comprehensive methods such as molecular karyotyping (microarray based comparative genomic hybridization, array-CGH), it will be easier to exclude maternal contamination and it will be possible to detect smaller deletions and duplications. Additionally, the widespread use of next-generation sequencing technologies can increase the detection rate of metabolic diseases, hemoglobinopathies and other single gene diseases which are also common causes of early intrauterine pregnancy loss (20). The use of new methods such as molecular karyotyping and interphase fluorescent in situ hybridization (iFISH) have allowed the evaluation of abortion materials in which conventional cytogenetic methods have failed.

The limited number of cases was the main limitation of the current study. From our point of view, the major reason for this limitation was the short sample collection period (5 years). An additional limitation factor of our study was maternal contamination which led to a low anomaly detection rate compared to the literature data

One of the most important complications in patients with a history of pregnancy loss is the risk of recurrence. Dealing with that can only be possible by assessing the causes of pregnancy loss. One of the most common way to define the reason is the examination of the abortion material to evaluate the possible chromosomal anomalies which helps the family to plan their future pregnancies.

Examination of the abortion material and determination of chromosomal anomaly in the recurrent pregnancy losses affects the genetic counseling process which will be given for the next pregnancies of the family. It is possible to reduce the frequency of maternal contamination by optimizing the chromosomal analysis technique in a way that does not change the yield time. In cases where it is not possible to examine the abortion material by chromosome analysis, the use of DNA-based molecular tests is an important alternative.

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*Conflict of Interest***:** The author have indicated no conflicts of interest regarding the content of this article.
Support and Acknowledgment: No financial support was received from any institution or person.
Researchers' Contribution Rate Statement: Concept/Design: PÖÇ; Analysis/Interpretation: PÖÇ, TAD; Data Collection: PÖÇ, FNÖ, TAD; Writer: PÖÇ; Critical Review: PÖÇ, FNÖ; Approver: PÖÇ, FNÖ

Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of the Haseki Training and Research Hospital (decision no: 2021/89, date: 06.10.2021).
Informed Consent: All participants gave written informed consent.

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