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Original scientific article

CYTOGENETIC ASPECTS OF MISCARRIAGE

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Chromosomal aberrations account for approximately 50% of fetal losses prior to the 15th week of gestation. The aim of this study was to determine the differences in frequencies and distribution of chromosomal aberrations in sporadic and habitual abortions. During a seven year period (2007.-2014.), we have analyzed 380 samples of chorionic villi after missed abortion in the Clinic for gynecology and obstetrics, Clinical center of Serbia. After first misscariage we analyzed 268 samples, and after habitual abortions 112 samples. For statistical analysis, we used χ^2 test. Karyotype analysis revealed chromosomal aberrations in 22,4% (85/380) of all samples. In the group after first abortion, we found an aberrant karyotype in 15,7% (42/268) of cases. In the group with habitual abortions, chromosomal aberrations were detected in 38,4% (43/112) of cases. Statistical analysis showed significant difference between these two groups, χ^2=11,34> $\chi^2_{(1\ I\ 0,05)}$=3,841i p<0,05. The distribution of chromosomal aberrations was similar in both groups. Also, in both groups, numerical chromosomal aberrations were the most common. The identification of cytogenetic causes is an important component in miscarriage etiology investigation, and it is recommended in order to improve genetic counseling of an involved couple.

Keyword: spontaneous abortion, habitual abortions, chromosomal aberrations

INTRODUCTION

One of the most common reproductive disorders is the spontaneous abortion (miscarriage), which occurs in 15-20% of all clinically recognized pregnancies. It is defined as the loss of pregnancy before the 20. gestational week (EL HACHEM *et al*., 2017). Between 2-5% of all couples suffer from habitual (recurrent) miscarriage, presenting two or more consecutive pregnancy losses (GABOON, 2013; CARP *et al*., 2001). Genetic factors are one of the main causes of spontaneous miscarriage. Cytogenetic analysis after first trimester abortions revealed chromosomal aberrations in 50% of cases, 86% of which were numerical, and 6% structural (ROBBERECHT *et al*., 2009). The most

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common chromosomal aberrations associated with miscarriage are aneuploidies, predominantly trisomies, monosomy X and polyploidies. More than 99% of all pregnancies with a pathological karyotype end in miscarriage before the 10. gestational week (STEPHENSON *et al*., 2002).

This study aims to determine if there are any differences in the frequency and distribution of chromosomal aberrations in sporadic and habitual abortions.

MATERIALS AND METHODS

This retrospective cohort study analyzed patients who were referred to the Laboratory of cytogenetics in the Clinic for gynecology and obstetrics, Clinical center of Serbia, during a seven year period, from 2007. to 2014. We have analyzed the karyotype of 380 samples of chorionic villi obtained after missed abortions in the first trimester (first 12 weeks of gestation). The samples were categorized into two cohorts, according to the number of previous abortions. The first group consisted of 268 samples obtained from women after first pregnancy loss (sporadic miscarriage). The second group comprised 112 samples obtained from women with a history of idiopathic habitual abortions (≥ 2 consecutive spontaneous miscarriage), without previous cytogenetic examinations. All of the enrolled cases had no identified causes for abortions. Cases of twin pregnancy, cases in which cell culture was failed, cases derived from parental chromosomal abnormality were excluded from the study.

Chorionic villi samples were collected from the placental tissue after curettage. After separation, villi were processed for direct culture analysis (24 hours incubation method), using standard laboratory procedure. Briefly, the villus samples were transferred into a sterile Petri dish containing 5 mL of RPMI media with 1-2 drops of colcemid (stock conc. 10 μg/mL). The villi were incubated for 24 hrs at 37°C. At harvest, the villi were treated with prewarmed 1% sodium citrate hypotonic solution for 20 minutes at 37°C. The villi were then fixed in a 3:1 methanol acetic acid mixture. After fixation, the villi were dissociated by a chilled 1:1 aqueous acetic acid solution and slides were prepared by a modification of SIMONI *et al*., (1983). The villus cell suspension was spread on a clean slide left at 37°C. The slides were aged for a time period of one to several days and subsequently GTG-banded for chromosome analysis. A minimum of 10 metaphases were analyzed in every sample. The International System for Human Cytogenetic Nomenclature was used to describe the karyotypes (ISCN, 2005).

For the statistical analysis we used χ^2 test of homogeneity and p value <0.05 was considered statistically significant.

RESULTS

Karyotype analysis of 380 samples of chorionic villi after missed abortions revealed chromosomal aberrations in 22,4% (85/380) of cases. In the sample group after sporadic miscarriage (group I), a pathological karyotype was detected in 15,7% (42/268) of cases. In the sample group after habitual abortions (group II), chromosomal aberrations were found in 38,4% (43/112) of cases. Statistical analysis showed a highly significant difference between the two groups, χ^2=11,34> $\chi^2_{(1 \text{ and } 0,05)}$=3,841 and p<0,05.

In the group with one abortion, the age of women ranged from 19 to 42 years, with a mean of 29,9 years, and in the group with repeated pregnancy loss the age of women ranged from 21 to 41 years, with a mean of 32,1 years.

The distribution of chromosomal aberrations did not show a statistically significant difference between the two analyzed groups. The obtained data has been expressed as the number of cases and percentage in the Tables 1. and 2. In both groups, numerical chromosomal aberrations were the most frequent finding, in 97,6% (41/42) of sporadic and 95,3% (41/43) of habitual abortions, respectively. The most common aberrations were aneuploidies, predominantly autosomal trisomies, accounting for 63,4% (26/41) of numerical aberrations in both groups.

Table 1. Numerical chromosomal aberrations in sporadic and habitual miscarriages

Chromosome aberration	Single miscarriage	Recurrent miscarriage
Trisomy 21	9 (21,9%)	8 (19,5%)
Trisomy 18	2 (4,9%)	7 (17,1%)
Trisomy 13	3 (7,3%)	2 (4,9%)
Trisomy 16	5 (12,2%)	8 (19,5%)
Trisomy 8	2 (4,9%)	1 (2,4%)
Trisomy 9	2 (4,9%)	/
Trisomy 2	2 (4,9%)	/
Trisomy 20	1 (2,4%)	/
Monosomy X	4 (9,8%)	4 (9,8%)
Polyploidy	7 (17,1%)	7 (17,1%)
sSMC	3 (7,3%)	3 (7,3%)
47,XXY	1 (2,4%)	1 (2,4%)
Total	41 (100%)	41 (100%)

sSMC-small supernumerary marker chromosome

Sex chromosome trisomies, monosomy X and polyploidies were found with the same frequency in both analyzed groups, in 2,4%, 9,8% and 17,1%, respectively.

In the first group (after first miscarriage) the most common findings were trisomy 21, trisomy 16 and polyploidy. In the second group (after habitual abortions) after trisomy 21 and trisomy 16, trisomy 18 was the most frequent finding.

Small supernumerary marker chromosome (sSMC) and structural chromosomal aberrations were detected with similar percentage in both groups.

Table 2. Structural chromosomal aberrations in sporadic and habitual miscarriages

Chromosome aberration	Single miscarriage	Recurrent miscarriage
45,XX,rob(13;14)(q10;q10)	1	/
45,XY,rob(13;22)(q10;q10)	/	1
46,XX,rob(13;14)(q10;q10)+13	/	1

DISCUSSION

The cytogenetic aspects of spontaneous abortions remain constant over the years, making karyotype analysis the "golden standard" in the detection of chromosomal aberrations causing this reproductive problem (JENDERNY, 2014).

The incidence rate of chromosomal aberrations in spontaneous miscarriage varies between 23% and 70.3% in different studies (ROBBERECHT *et al*., 2009; MENTEN *et al*., 2009; ZHANG *et al*., 2009; LOMAX *et al*., 2000; SOLER *et al*., 2017).

Thus, the overall incidence of 22,4% of chromosomal aberration in spontaneous abortions, found in our study, is comparable with literature data.

Nevertheless, in contrast to the previous studies showing no differences in the frequency of chromosomal aberrations in sporadic and habitual miscarriage, our investigation revealed a statistically significant difference between these two groups (DORIA *et al*., 2009; HORIUCHI *et al*., 2019).

Van den Berg et al. in their review comprised several studies and revealed a frequency of chromosomal aberrations in sporadic and habitual abortions of 45% (in 7012 samples) and 39% (in 1359 samples), respectively (VAN DEN BERG *et al*., 2012). In the study of Nikitina et al., the frequency of embryos with abnormal karyotypes was shown to be significantly higher in sporadic abortions compared to repeated miscarriage (56.7 and 46.6%, respectively) (NIKITINA *et al*., 2016).

In our study, a significantly higher incidence of chromosomal aberrations was found in habitual abortions.

The proportions of chromosomal aberrations causing miscarriage were nearly equal in both studied groups in our investigation. The most frequently detected were aneuploidies, predominately autosomal trisomies, which are the result of de novo chromosomal nondisjunction in parental mejosis (SIERRA and STEPHENSON, 2006).

Despite the fact that most of the studies revealed structural chromosomal aberrations as the main cause of recurrent pregnancy loss, some studies detected predominantly numerical chromosomal aberrations in habitual abortions (MARQUI, 2018). These differences may result from the concept of the study, predominantly different sample including criteria. The higher frequency of trisomies 16, 18 and 21, in our study, is in line with the evidence available from the literature (CARP *et al*., 2001; GONCALVES *et al*., 2014).

It has been shown that couples, which have had a spontaneous abortion due to an aneuploidy, have a higher risk for trisomy recurrence in the next pregnancy. In these cases there are two possible causes. Firstly, there is a possibility of a genetic mistake in gene control of chromosomal disjunction in mejosis, and secondly, gonadal mosaicism in one parent (GABOON, 2013; TURNPENNY, 2011).

Cytogenetic studies of unfertilized ova showed a high percentage of aneuploidies (about 13%), caused by a mistake in maternal mejosis. Direct analysis of uncondensed sperm nucleuses using the fluorescent hybridization method, revealed a high incidence of chromosomal aberrations in men having history of habitual abortions (RUBIO *et al*., 1999).

CONCLUSIONS

Repeated spontaneous abortions are still a challenging reproductive disorder for the patients, as well as doctors. Despite the fact that many factors can lead to miscarriage, the role of

chromosomal aberrations in this reproductive problem remains constant over the years. The identification of cytogenetic causes is an important component in miscarriage etiology investigation, and it is recommended in order to improve genetic counseling of the involved couple.

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CITOGENETIČKI ASPEKTI POBAČAJA

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Izvod

Citogenetičke analize pokazale su da je 50% pobačaja u prvom trimestru trudnoće uzrokovano hromozomskim aberacijama. Ova studija ima za cilj da utvrdi postoje li razlike u frekvenciji i distribuciji hromozomskih aberacija u sporadičnim i habitualnim pobačajima. U periodu od sedam godina (2007.-2014.g.), na Klinici za ginekologiju i akušerstvo Kliničkog centra Srbije, analiziran je kariotip 380 uzoraka horionskih resica nakon zaostalog ("missed") pobačaja u prvom trimestru trudnoće. Nakon prvog pobačaja analizirano je 268 uzoraka, dok je 112 uzoraka analizirano u slučajevima dijagnoze habitualnih pobačaja. Za statističku obradu podataka korišćen je χ^2 test homogenosti. Analizom kariotipa 380 uzoraka horionskih resica nakon zaostalih pobačaja, hromozomske aberacije nađene su u 22,4% (85/380) slučajeva. U grupi uzoraka nakon jednog pobačaja, patološki kariotip je detektovan u 15,7% (42/268) slučajeva. U grupi uzoraka nakon habitualnih pobačaja, patološki kariotip je nađen u 38,4% (43/112) slučajeva. Statističkom obradom podataka pokazano je da postoji statistički visoko značajna razlika između ove dve grupe, $\chi^2=11,34 > \chi^2_{(1\ 1\ 0,05)}=3,841$ i $p<0,05$. Distribucija hromozomskih aberacija bila je približno jednaka u obe ispitivane grupe. U obe grupe najzastupljenije su bile numeričke hromozomske aberacije. Identifikacija citogenetičkih uzroka pobačaja predstavlja važnu komponentu u ispitivanju etiologije, i preporučuje se, jer svaki dobijeni rezultat može da olakša genetičko savetovanje pogođenog para.

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