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Cytogenetic Evaluation of Couples With Spontaneous Abortion, Still Birth and Recurrent Miscarriage in Qazvin: Report and Review

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Abstract

Background: Chromosomal abnormality plays an important role in different types of miscarriages.
Objectives: The present study was designed to investigation chromosomal anomalies in three groups of couples with recurrent abortion (RA), spontaneous abortion (SA) and still birth (SB).
Patients and Methods: In this retrospective study, the frequency of chromosomal aberrations was investigated among 260 couples with miscarriage, which had referred to the cytogenetic section of a reference laboratory in Buali hospilal, Qazvin, Iran from 2009 to 2014. Metaphase spreads were analyzed using G-banding.
Results: In this study, 7.6% of couples had chromosomal aberrations including, balanced reciprocal translocations, robertsonian translocations, inversions and sex chromosome aneuploidy. Frequency of balanced translocations was higher, specifically in couples with SA.
Conclusions: In this investigation we showed that chromosomal abnormalities could be one of the important causes of miscarriages. Cytogenetic evaluation of couples, which experienced different types of miscarriage, may prevent unnecessary treatments.

Keywords: Recurrent Abortion, Spontaneous Abortion, Still Birth, Chromosome Abnormality

1. Background

Spontaneous abortion (SA) is the loss of the fetus before 20 weeks of gestation, still birth (SB) is often fetal death after 20 weeks of gestation, and recurrent abortions (RA) is defined as three or more consecutive pregnancy losses before 24 weeks of gestation (1). The causes of SA, SB and RA are heterogeneous, including endocrine dysfunction, auto immune disorders, genetic abnormalities, maternal and paternal age, infectious diseases, environmental toxins and congenital or structural uterine anomalies etc. (2). Chromosomal abnormalities are common among couples with reproductive problems and different types of miscarriage (3).

Several cytogenetic investigations have been performed in various countries to determine the pattern of chromosome abnormalities in parents with fetal wastage. They estimated that frequency of parental chromosomal abnormality (specially structural chromosomal aberrations) is 2% to 8% (4).

Therefore, cytogenetic study of parents with a history of miscarriage is an integral part of diagnostic clarification. Several other studies have shown that the most common structural chromosomal aberrations among parents with different types of miscarriage are balance translocation and inversion (5). During the gametogenesis stage, unequal cross over in meiosis of prophase causes duplication and deletion in some gametes of these people. Clinically, this phenomenon causes the death of the developing embryo and spontaneous miscarriages (5). Different studies in Iran have reported cytogenetic evaluation of parents with recurrent miscarriage (6-8). However, to the best of our knowledge, no such study has been done to compare cytogenetic abnormalities among parents with SA, SB and RA.

2. Objectives

The aim of the present study was to compare the types of chromosomal aberration among parents with SA, SB and RM, who were referred to our genetic clinic, and to review the related literature.

3. Patients and Methods

This retrospective study was done on 260 couples with SA, SB and RA referred to our cytogenetic clinical laboratory from 2009 to 2014. The mean age of these individuals was 35.1 $\pm$ 3.7, 34.4 $\pm$ 4.1 and 34.9 $\pm$ 3.9, respectively

and all of them had no children before. For chromosome analysis, firstly, lymphocyte cultures were set up in the laboratory by adding 0.5 mL of heparinized blood to 4.5 mL of complete medium RPMI-1640 (Sigma, St. Louis, MO, USA) supplemented with 1% L-Glutamine (20 mM, Sigma), 15% fetal calf serum (Gibco-BRL, Paisley, UK), penicillin (100 U/uL) and streptomycin (100μg/uL), followed by the addition of phytohemagglutinin as a mitogen (PHA, 1%) (Sigma, St. Louis, MO, USA). Cells were incubated for 72 hours in a 5% CO_2 incubator. Seventy-two hours after culture initiation, colcemid (Sigma, St. Louis, MO, USA) at a final concentration of 4 μg/mL was added to the cultures. The cultures were then centrifuged at 1,200 rpm for 10 minutes. The pellet was resuspended in hypotonic solution (KCl, 0.075 M, Merck, Darmstadt, Germany) and after 30 minutes, centrifuged at 1,200 rpm for 10 minutes, then resuspended in freshly prepared, ice-cold fixative containing methanol: acetic acid (3: 1) (Merck, Dar mstadt, Germany), and left for 20 minutes at room temperature. The solution was then centrifuged at 1200 rpm for 10 minutes, and the pellet was resuspended in freshly prepared ice-cold fixative containing methanol: acetic acid (6: 1). If the solution was not clear after additional centrifugation, the last step was repeated until a clear solution was obtained. After decantation to reduce the volume to about 0.2 mL, the pellet was mixed with the remaining fixative and dropped from about 3 cm with a Pasteur pipette onto an ethanol-washed slide; the fixative was removed by slight blowing, decantation and air-drying. Subsequently, the slides were stained in 5% Giemsa solution for 10 minutes. G banding using trypsin and Giemsa (GTG banding) was used for cytogenetic analysis with resolution of 400 - 450 bands. Nucleolar organizing regions (NOR) banding and C-banding was also done to confirm the satellites on acrocentric chromosomes and heterochromatin regions respectively, wherever necessary (9). For the cytogenetic analysis, twenty-five metaphases were analyzed in all patients but in cases that had abnormalities and mosaicism, metaphase analysis was performed on 60 metaphases. Chromosomal abnormalities were reported according to the current International Standard Nomenclature (ISCN) (10).

3.1. Statistical Analysis

Statistical analysis including mean, standard deviation (SD) and correlation coefficients (R) were done using the Prism (version 3) software. Additionally, One-Way analysis of variance (ANOVA) was carried out to determine significant differences between the studied groups. $P < 0.05$ were considered statistically significant.

4. Results

In this study, the mean age of male partners was 34.3 ± 0.99 and for female partners was 28 ± 0.88. In total, 260 couples were investigated. Among these, 144 couples had RA, 66 couples had SA and 40 couples had SB. About 144 couples with RA (49%) had two miscarriages and 10% had three miscarriages, and the remaining (41%) had four or more miscarriages. Furthermore, 6.9% of RA patients had total chromosomal abnormality (structural and numerical). Among the couples with SA and SB, 9% and 10% had chromosomal aberrations, respectively (structural and numerical) (Almost Almost all of the couples in the three groups showed structural aberrations, and only 10% of patients with RA had numerical abnormality (Tables 2, 3). Frequency of reciprocal translocation was significantly higher in patients with SA compared to patients with RA and SB ($P < 0.05$) (Figure 1). In patients with SB, robertsonian translocation was not seen, but 20% and 16% of couples with RA and SA had robertsonian translocation, with no significant difference in this regard ($P > 0.05$). Inversion frequencies in RA and SB couples were 30% and 50%, yet in the SA patients we didn't see this structural abnormality. Ten percent and 16% of patients with RA and SA had chromosomal deletions, yet in couples with SB we did not see this aberration. Only 10% of couples with RA had numerical changes, and in others, these changes were not seen. Reciprocal translocation was significantly higher in SA patients in comparison to other groups. Regarding the other chromosomal aberrations frequency, we did not see significant differences ($P > 0.05$).

5. Discussion

Chromosomal abnormality is involved in most spontaneous abortions (23). Approximately 50% of first trimester, 35% of second trimester and 11% of third trimester of pregnancies have genetic abnormality; this genetic abnormality is usually associated with fetal and parental chromosomal aberrations (24, 25). In this investigation, couples experiencing RA had the highest frequency in comparison to couples with SA and SB, yet the rate of chromosomal abnormality in these three groups was nearly the same. Generally, 68% of phenotypically normal couples with balanced translocations have reproductive problems and in these couples the risk of miscarriage is approximately doubled (5). In a way that balanced translocations are seen in 4-8% of couples with RA (25). Also, in our study, the frequency of balanced translocations was higher in comparison to other chromosomal abnormality, specifically reciprocal translocation in couples with SA (Table 2 and Figure 1).

Table 1. Frequency of Total Chromosomal Abnormality

Value	Sample Number	Frequency of Chromosomal Aberrations, (%)
Recurrent abortion	144	6.9
Spontaneous abortion	66	9
Still birth	40	10

Table 2. Frequency of Different Types of Chromosomal Abnormality

Value	Recurrent Abortion, (%)	Spontaneous Abortion, (%)	Still Birth, (%)
Reciprocal translocation	30	66	50
Robertsonian translocation	20	16	-
Inversion	30	-	50
Deletion	10	25	-
Aneuploidy	10	-	-

Table 3. Structural Chromosomal Abnormalities Identified Among the Studied Groups

Number of Sample	Abnormalities	Gender
Recurrent Abortion		
1	46, XX, t (10; 13) (q 21; q 33)	F
2	46, XY, t (12; 18) (q 15; q 11.2)	M
3	46, XY, t (4; 7) (q 31.2; q 23)	M
4	45, XX, rob (13; 14)	M
5	45,XX, rob (13; 14)	F
6	46, XX, inv (9) (p 11; q 13)	F
7	46, XX, inv (Y) (p 11; q 11.23)	F
8	46, XX, inv (9) (p 11; q 13)	F
9	46, XY/ 47, XYY	M
10	46, XX/ 46, XX del (17) (q)	F
Spontaneous Abortion		
1	46, XY, t (1; 3) (q 12; q 29)	M
2	46, XY, t (7; 14) (q 33;q 32.3)	M
3	46, XX, t (8; 10) (q 24.2; q 25.2)	F
4	45, XY, t (13; 15)	M
5	46, XX, del (Xq)	F
6	45, XX, rob (13; 14)	F
Still Birth		
1	46, XX, t (16; 21) (p 10;q 10)	F
2	46, XX, t (10; 13) (q 21; q 33)	F
3	46, XX, inv (9) (p 11; q 13)	F
4	46, XX, inv (5) (p 13; q 13)	F

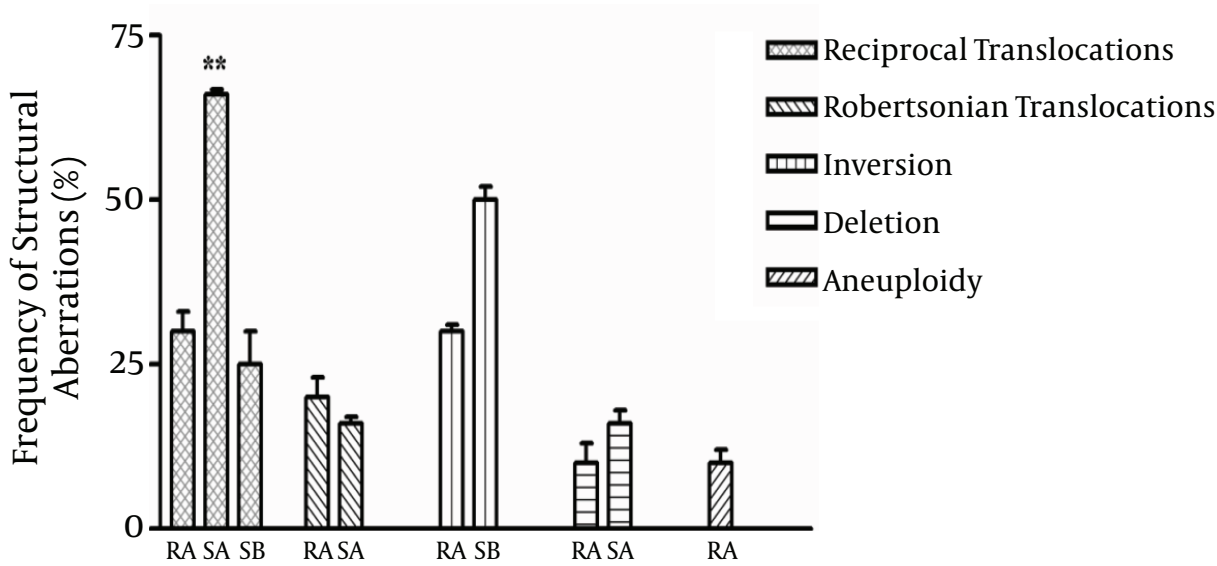

Figure 1. Different Abnormality Rate in Various Studied Groups

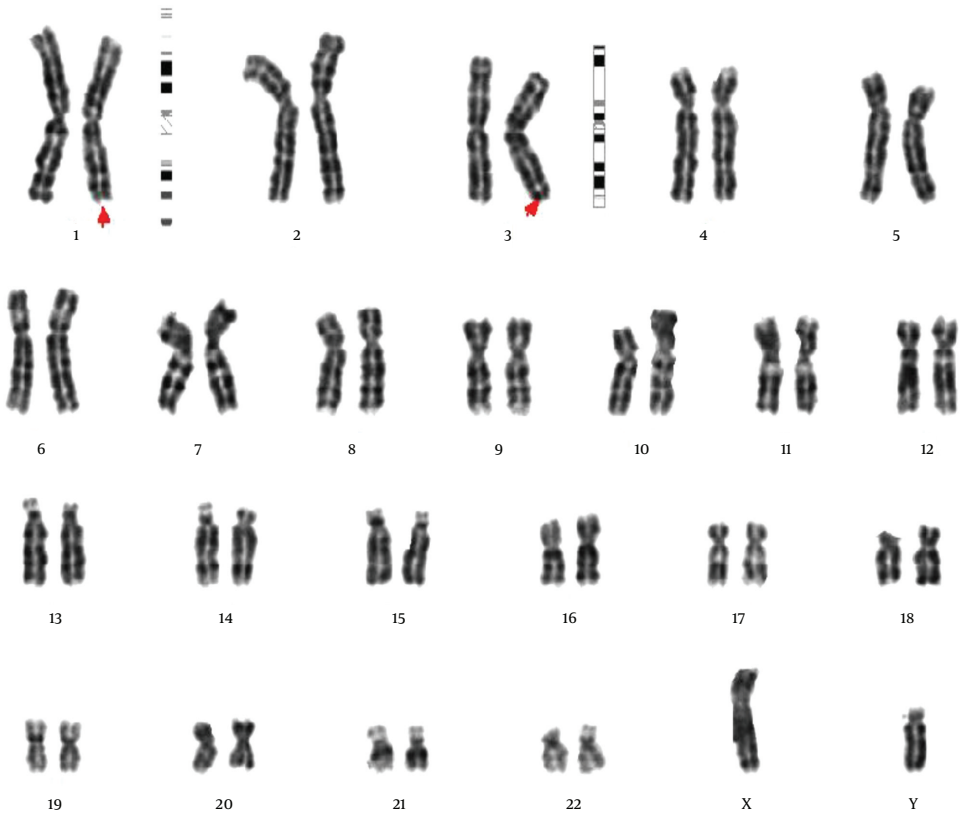

Figure 2. Abnormal Male Karyotype With Balanced Reciprocal Translocation Between Long Arms of Chromosomes One and Three; 46, XY, t (1; 3) (q 42; q 29)

Table 4. Review of Literature Regarding Different Types of Miscarriage

Author	Total couples	Total Abortion	Structural	Numerical
Recurrent Miscarriage				
Ghazaey et al. (11) (2015)	728	11.7	7	0.9
Gaboon et al. (12) (2014)	125	12	12	-
Choi et al. (13) (2014)	86	7.4	1.4	8
Ocak et al. (14) (2013)	495	5.7	92.9	7.1
Akgul et al. (15) (2009)	90	9.9	3	8.3
Espino et al. (16) (2008)	916	2.76	2.28	0.47
Mozdarani et al. (17) (2008)	110	9.5	-	-
Elghezal et al. (4) (2007)	1,400	6.9	6	4.9
Reddy et al. (18) (2005)	742	4	2.9	1.2
Present research	260	6.9	3	0.3
Spontaneous Abortion				
Yakut et al. (19) (2015)	382	33.24	3.6	28.8
Rabieqa et al. (20) (2015)	47	72	-	72
Choi et al. (13) (2014)	164	50.6	7.2	20
Bastos et al. (21) (2014)	333	27.3	-	92.3
Alonso et al. (22) (2011)	120	65	-	100
Present research	66	9	7	-

In this regard it had been shown that balanced translocations cause meiotic blocking of spermatogenesis, yet ovogenesis is conserved and produces gametes with unbalanced forms of chromosomal anomaly (4); our results confirmed this phenomenon, because the rate of balanced translocation was more in females than in males.

In our research, one couple had a familial marriage, including a 30-year-old man and a 28-year-old woman (cases No 4 and 5) (Table 3). This couple had four miscarriages, before 3 months. Karyotype analysis results showed that both of them had a robertsonian translocation between chromosomes 13 and 14 (Figure 2). However, chromosomal analysis of families of the couple was unknown, and they did not cooperate with us. Prenatal diagnosis was strongly recommended for them, because there was 30% chance of Patau syndrome that will be inherited in every future generation from this family.

In this study, reviewing different recent researches showed that the average chromosomal abnormality frequencies among the couples with RA and SA in different countries were 7.7% and 49%, respectively (Table 4). Our study showed that these frequencies were 6.9% and 9% for RA and SA. Regarding RA, our results were close to the average of other countries' frequency. However, in couples with SA, our results showed that the rate of chromosomal aberrations was less than other countries (Table 4). Usually using different sample sizes and criteria for investigation of cases, results in variable prevalence. As far as we reviewed, studies about the chromosomal abnormality frequency of SB miscarriage in different countries were on the fetus, rather than their parents. In this regard, our study was on couples with SB, and 10% of them showed chromosomal abnormality, specifically reciprocal translocation and inversion. Numerical chromosomal abnormality usually occurs with a low frequency (< 0.15% of cases) (5). Our results confirmed this, because only 10% of couples with RA showed sex chromosome aneuploidy. In SA and SB cases, we did not see numerical chromosomal abnormality. In conclusion, our investigation showed that chromosomal abnormalities are common among couples having miscarriages. Frequency of these chromosomal aberrations does not show significant difference among the couples with RA, SA and SB. Therefore, it would be reasonable to recommend chromosome analysis to these couples.

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Footnotes

Authors' Contribution: Reza Najafipour: head of cytogenetic lab; Javad Ansari: data analysis; Manijeh Jalilvand: cell culture and harvesting; Sahar Moghbelinejad: head of the genetic lab.

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