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Original Article

Cytogenetic screening in couples with Habitual Abortions

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ABSTRACT

Objective: Habitual abortion (HA) is defined at least three consecutive pregnancy losses. One of the etiologic causes is parental chromosomal anomalies. In this study, we aimed to that investigate the effect of parental chromosomal abnormalities on HA.
Methods: The cytogenetic results of patients with at least three abortions referred to our university hospital between January 2010 - March 2017 were evaluated. A total of 1154 couples with HA were analysed. Peripheral lymphocyte cultures incubated for 72 h were used for karyotype analysis via the Giemsa banding technique.
Results: Of a total 1154 couples (2308 patients) 37 female (3.2%) and 17 male (1.47%) had abnormal karyotypes. Reciprocal translocation carriage (n = 26; 1.12%) was the most commonly detected structural anomaly, followed by X chromosome mosaicism (n = 16; 0.69%),Robertsoniantranslocation (n = 9; 0.38%), Chromosomal inversion (n = 6; 0.26%). Chromosomal polymorphisms, which are considered minor chromosomal changes, were detected in 221 (9.57%) individuals.
Conclusion: Our study exhibits that chromosomal analysis in patient with HA is an appropriate approach to elucidate the aetiology of HA. Data from cytogenetic screening can be used in guiding couples planning future pregnancies and in prenatal diagnosis of chromosomal anomalies in the foetus.

Introduction

Habitual abortion (HA) is defined as pregnancy loss occurring before 20 weeks of gestation in 3 or more pregnancies [1,2]. It is a common obstetric problem that concerns both patients and obstetricians. Research has suggested that it affects approximately 2% of all women of reproductive age worldwide [3,4]. Large number of aetiological factors, such as uterine abnormalities, endocrine problems, immunological abnormalities, inherited thrombophilia and infectious disease, have been proposed to be associated with HA [5,6]. In women with HA but not the above-mentioned factors, studies have suggested that balanced chromosomal abnormalities in parents are one of the main reasons for HA [7,8].

In cytogenetic analyses of couples with HA, balanced chromosomal translocation carriage, which can be fatal for the development of the embryo or foetus, was detected at a rate of 2–8% [9,10].

Studies have shown that there is generally a history of abortion in couples with an abnormal karyotype due to the formation of unbalanced chromosomal material, such as duplication or deletion of the products of conception [7,11]. In such cases, screening chromosomal anomalies and providing appropriate counselling may be helpful in the prevention of adverse outcomes in future pregnancies.

In this retrospective study, we aimed to assess types of parental chromosomal anomalies and their prevalence in couples with a history of HA who were referred to our genetic clinic.

Material and method

The study included 1154couples (2308 individuals) who had a history of 3 or more spontaneous abortions before 20 weeks of gestation and presented to the Obstetrics &Gynecology Department of Harran University, Medicine School between January 2010 and March 2017. Couples with antiphospholipid syndrome, inherited thrombophilia, uterine anomalies and endocrine diseases were excluded. Patients with a history of previous live childbirth and IVF (in vitro fertilization) pregnancies were included

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in the study, and those with a history of giving birth to a child with a foetal anomaly were excluded.

In all patients included, 5 ml of venous blood were drawn in heparin tubes for chromosomal analysis. To obtain a sufficient number of metaphases, peripheral lymphocytes were incubated for 72 h at 37 °C. The preparations obtained were stained with the Giemsa banding technique. A modified macro-culture technique was used for chromosomal analysis [12]. A minimum of 30 metaphases with 450 band resolution were evaluated under the microscope to determine numerical and morphological chromosomal anomalies. The number of metaphases evaluated was increased to 50 in cases of suspicious of mosaicism and abnormal chromosomes. İn order to confirm the anomaly in the presence of any chromosomal anomaly, especially komplex translocations, the fluorescence in situ hybridization (FISH) technique was also used. In accordance with the International System of Human Chromosomal Nomenclature [9], the chromosomal anomalies were classified into the following subgroups: structural chromosomal anomalies, mosaicism and chromosomal polymorphisms.

A chromosomal polymorphism was defined as the detection of increased heterochromatin domains in chromosomes 1, 9, 16 and the Y chromosome, pericentric inversion of chromosome 9 and satellite variants in acrocentric chromosomes.

Results

Of the 1154 couples included, a structural chromosome anomaly was detected in 55 (2.38%) (Table 1). Reciprocal translocation carriage (n = 26; 1.12%) was the most commonly detected structural anomaly, followed by gonosomalmosaicism (n = 16; 0.69%) Table 2 summarises the distribution of the chromosomal anomalies according to gender, indicating female predominance.

Of the reciprocal translocations detected, the chromosome 9 anomaly was the most commonly observed anomaly among women, whereas the chromosome 3 anomaly was most common among men. A Robertsonian translocation was observed between chromosomes 13 and 14 [(45, XX, rob [13,14] (q10;q10)] in both genders.

Table 2
Distribution of chromosomal anomalies according to gender.

Chromosomal anomalies	Female, n (%)	Male, n (%)	Overall total, n (%)
Reciprocal translocation	15 (1.29)	11 (0.95)	(1.12%)
Robertsonian translocation	5 (0.43)	2 (0.17)	7 (0.30)
Chromosomal inversion	4 (0.34)	2 (0.17)	6 (0.25)
Gonosomalmosaicism	14 (1.21)	2 (0.17)	16 (0.69)
Total	**38 (3.29)**	**17 (1.47)**	**55 (2.38)**

Chromosomal polymorphisms were detected in 221 individuals (9.57%) in the study population. The most common polymorphism was 1qh+ (n = 44; 1.91%); followed by 9qh+ (n = 32; 1.38%). Table 3 presents the polymorphism types and the number of individuals with these polymorphisms. No combined chromosomal anomaly was detected in any of couples screened.

Discussion

Parental cytogenetic anomalies are important aetiological factors in couples with a history of HA, the prevalence of which varies from 3% to 11% [8]. The aim of parental karyotyping is to identify individuals with a normal phenotype but carrying a chromosomal anomaly and to minimise the rates of pregnancy losses and malformed births, which may result from gametes with an abnormal karyotype [7,9].

The role of parental chromosomal anomalies in HA has long been known [7,10]. In individuals with a chromosomal rearrangement, gametes harbouring an unbalanced chromosomal structure with a duplication or a deletion can occur due to disorders of pairing up or disjunction during meiosis [10,11]. Adverse outcomes, such as HA, foetal loss or a malformed child, may occur in pregnancies resulting from fertilization of these gametes with an abnormal structure [11,13].

Pre-implantation Genetic Diagnosis (PGD) is suggested to avoid (another) miscarriage andother adverse outcomes in couples with

Table 1
Chromosomal abnormalities in individuals with a major chromosomal anomaly.

Chromosomal anomalies	Female carriage	Male carriage
Reciprocal translocation	46,XX,t(10,18)(q11;q11)(n = 4)	46,XY,t(2,8)(q25;q13)(n = 3)
	46,XX,t(9,20)(q21;p11.2)(n = 3)	46,XY,t(3,11)(q22;q22)(n = 2)
	46,XX, t(4,11)(p13;q22.3)(n = 2)	46,XY,t(3,5)(q12;p12)
	46,XX,t(6,7)(p21;q36)	46,XY,t(3,11)(q21;q22)
	46,XX,t(9,12)(p14;q24.3)	46,XY,t(5,10)(q24;p15.3)
	46,XX,t(9,16)(q22.1;p11.1)	46,XY,t(8,22)(q22;q13)
	46,XX,t(5,8)(q15;q21.2)	46,XY,t(3,7)(q27;q21)
	46,XX,t(1,14)(q21;q32)	46,XY,t(14,15)(q11.2;q2)
	46,XX,t(14,15)(q11.2;q24)	
Robertsonian translocation	45,XX,rob(21,22)(q10;q10)	45,XY,rob(21,22)(q10;q10)
	45,XX,rob(13,14)(q10;q10)	45,XY,rob(13,14)(q10;q10)
	45,XX,rob(13,14)(q10;q10)	
	45,XX,rob(13,14)(q10;q10)	
	45,XX,rob(13,14)(q10;q10)	
Chromosomal inversion	46,XX,inv(16)(p11;q11)	46,XY,inv(9)(p22;q13)
	46,XX,inv(15)(p11;q12)	46,XY,inv(12)(p11;q13)
	46,XX,inv(12)(p11;q14)	
	46,XX,inv(8)(p12;q24)	
Gonosomalmosaicism	mos 47,XXX[46]/46,XX[4](n = 2)	mos 47,XXY[47]/46,XY[3]
	mos 47,XXX [47]/(46,XX [3](n = 2)	mos 47,XXY[44]/46,XY[6]
	mos 47,XXX[3]/46,XX[47]	
	mos 47,XXX[4]/46,XX[46]	
	mos 47,XXX[5]/46,XX[45]	
	mos 45,X[5]/46,XX[45](n = 2)	
	mos 45,X[3]/46,XX[47]	
	mos 45,X[4]/46,XX[46]	
	mos 45,X[6]/46,XX[44](n = 3)	

Table 3
Prevalence of polymorphic variants in heterochromatin and satellites in 221 couples with recurrent abortions.

Polymorphic variants in heterochromatin	No. Patient with CA (%)	Polymorphic variants in satellite	No. Patient with CA (%)
1qh+	44 (1.91)	13ps+	21 (0.90)
9qh+	32(1.38)	14ps+	17 (0.73)
16qh+	30 (1.29)	15ps+	13 (0.56)
Yqh+	22 (0.95)	21ps+	12 (0.51)
inv(9)	19 (0.82)	22ps+	11(0.47)
Total	**147 (6.36)**	**Total**	**74(3.20)**

Abbreviation: CA: chromosomal aberrations.

a structural chromosomal abnormality in one of the partners and thus to increase the chance of an ongoing pregnancy. It can also be offered as an alternative to traditional prenatal diagnosis (PND) with possible pregnancy termination in case of an abnormal result. The main aims of PGD include: offering women at risk of having children with genetic abnormalities the widest possible range of choices; providing reassuranceandre ducing the anxiety associated with reproduction, especially among high-risk women; and enabling continued pregnancies of women at high risk by confirming the absence of certain genetic diseases [14,15].

It has been suggested that the frequency of chromosomal anomalies is 0.3–0.4% in the general population [16]. However, the frequency of chromosomal anomalies was 3–11% in screening studies conducted in couples with a history of HA [8,11,13,17]. In our study, the incidence of structural chromosomal anomalies (2.47%) was within the range reported in the literature.

The most common chromosomal abnormalities that are associated with HA were balanced reciprocal and Robertsonian translocations and chromosomal inversions [16,18]. These translocations result from the rearrangement of segments between nonhomologous chromosomes that is not linked to either an increase or a depletion of genetic material. As reported previously, the first clinical symptom may be spontaneous abortion caused by an unbalanced chromosome structure in the originating gametes [11], although no phenotypic pathology is observed in individuals carrying balanced chromosome translocations [13,19]. In our study, the most commonly observed structural chromosomal anomaly was a reciprocal translocation, which was observed in 27 (2.33%) couples with HA who were screened.

Gonosomal mosaicism, another cytogenetic disorder characterised by an altered karyotype with a different number of sex chromosomes in the cells of an organism, has been observed in couples with HA [13,16,17,20]. Because of the fact that embryonic lethality and problems in the development of oocytes, gonosomal mosaicism can cause HA [18,20,21]. In a study of 336 couples with HA, the prevalence of sex chromosome mosaicism was 3.57% [9]. In our series, gonosomal mosaicism was detected in 16 (1.38%) of the 1154 couples with HA. This inconsistency is attributed to the larger sample size in our study.

İn present study, 47 XXX / 46 XX, 45 X / 46XX and 47XXY / 46 XY gonodalmosaicism types were determined.Spontan miscarriage in women with 47 XXX / 46 XX can be explained by genomic inbalance caused by genes escaping from X chromosome inactivation in embryos with more than two X chromosome.

To exclude 45X / 46 XX mosaicism related to advanced women age; the age of women with this mosaicismwas examined. All women were younger than 28 years old and so this mosaicisms were attributed to congenital genetic disorders

Since men with 47XXY/46XY mosaicism were azoospermic, pregnancies were obtained with IVF. Hovewer they refused PGD and these pregnancies resulted in spontan miscarriages

A chromosomal polymorphism is defined as a minor chromosomal change in a normal karyotype, and there is controversy regarding the effects of such polymorphisms on reproductive function. It has been proposed that these alterations are not associated with HA because they are located at repeating DNA regions harbouring no genes [22]. However, some authors have suggested that these polymorphisms are associated with HA, as they play a major role in cell division and chromosome movement and change synapsis between homologous chromosomes during meiosis [23]. The most common minor chromosomal variants include increased heterochromatin domains in chromosomes 1, 9, 16 and the Y chromosome, pericentric inversion of chromosome 9 and satellite variants in acrocentric chromosomes [11]. In a chromosomal analysis of couples with HA, the prevalence of a heterochromatin polymorphism was 1.9–15.82% and the prevalence of a satellite polymorphism was 22.15% [11,24,25]. The frequency of heterochromatin polymorphisms in our study is in agreement with the literature, but the frequency of satellite polymorphisms is lower. The variation is attributed to differences between the laboratories used to assess the satellite polymorphisms.

In one cytogenetic study of couples with HA, the frequency of chromosomal translocations was higher among women when compared to men [9]. It has been proposed that an unbalanced karyotype in the sperm cells of men carrying chromosomal translocations more frequently lead to infertility disorders through gametic selection [16].

The risk of foetal malformation, a mentally retarded child and spontaneous abortion is increased in subsequent pregnancies in the presence of a parental chromosomal anomaly in couples with HA [7,9]. Thus, cytogenetic screening during the evaluation of HA is important to decrease negative obstetric outcomes in subsequent pregnancies [9,10]. Counselling couples with HA affected by chromosomal anomalies may aid in the planning of future pregnancies, in addition to screening of chromosomal anomalies in the subsequent pregnancy [10]. In our study, all the couples with a detected chromosomal anomaly received genetic counselling and information regarding prenatal screening methods for subsequent pregnancies.

In conclusion, the present study showed that the frequency of chromosomal anomalies is higher in couples with a history of HA compared to the healthy population. This finding is important because it indicates that chromosomal anomalies may be one of the aetiological factors responsible for HA. Therefore, parental cytogenetic studies may be an ancillary diagnostic tool in fertility disorders during the evaluation of the aetiology of HA.

Conflict of interest

The authors have stated explicitly that there are no with this any financial support or relationships that may pose potential conflict of interest in this article.

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