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ANALYSIS OF POLYMORPHIC CHROMOSOME VARIANTS IN COUPLES WITH RECURRENT PREGNANCY LOSS

TEKRARLAYAN GEBELİK KAYBI OLAN ÇİFTLERDE POLİMORFİK KROMOZOM VARYANTLARININ ANALİZİ

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ABSTRACT

Objective: This study evaluated chromosome polymorphisms (1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+ and Yqh+) in a case group (n=1688) with two or more recurrent pregnancy losses (RPL) and a control group (n=80).

Materials and Methods: The control group was selected from 40 married couples who had no known hereditary disease, were not relatives, had healthy children, and had no history of miscarriage and/or stillbirth. Phytohemagglutinin-induced peripheral blood lymphocytes were cultured for 72 h. The Giemsa–Trypsin–Leischman (GTL) banding technique was applied to the obtained metaphase plates; thirty metaphase plates were examined at the 450-550 band level in each individual.

Results: A total of 488 individuals in the case group and 13 in the control group carried polymorphic chromosome variants.9qh+ chromosome polymorphisms were more prevalent in the case group than in the control group (p=0.028). Other variants were also increasingly observed in the case group (p=0.014).

Conclusion: Our findings reveal a potential relationship between RPL and chromosome polymorphisms. Karyotype analysis and appropriate genetic counseling increase the chance of having healthy children in individuals with RPL.

Keywords: Recurrent pregnancy loss, cytogenetics, chromosome polymorphisms

ÖZ

Amaç: Bu çalışmada, iki veya daha fazla tekrarlayan gebelik kaybı (TGK) olan vaka grubu (n=1688) ve kontrol grubu (n=80) kromozom polimorfizmleri (1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+ ve Yqh+) açısından değerlendirildi.

Gereç ve Yöntem: Kontrol grubu bilinen bir kalıtsal hastalığı olmayan, akraba olmayan, sağlıklı çocuğu olan, düşük ve/veya ölü doğum öyküsü olmayan 40 evli çiftten seçildi. Fitohemaglutinin ile indüklenmiş periferik kan lenfositlerinin 72 saatlik kültürü yapıldı. Elde edilen metafaz plaklarına Giemsa-Trypsin-Leischman (GTL) bantlama tekniği uygulandı. Her birey için yapılan kromozom analizinde 450-550 bant seviyesinde 30 metafaz plağı incelendi.

Bulgular: Polimorfik kromozom varyantı taşıyan bireylerin sayısı vaka grubunda 488 ve kontrol grubunda ise 13 olarak belirlendi. 9qh+ kromozom polimorfizminin kontrole göre vaka grubunda daha yüksek oranda olduğu belirlendi (p=0,028). Bununla birlikte, diğer bazı varyantların da vaka grubunda artış eğilimi gösterdiği gözlendi (p=0,014).

Sonuç: Bulgularımız TGK ile kromozom polimorfizmleri arasında bir ilişki olabileceğini kanıtladı. TGK olan bireylerde karyotip analizi ve uygun genetik danışma sağlıklı çocuk sahibi olma şansını artırmaktadır.

Anahtar Kelimeler: Tekrarlayan gebelik kaybı, sitogenetik, kromozom polimorfizmleri

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INTRODUCTION

Recurrent pregnancy loss (RPL), or habitual abortion, is the spontaneous termination of two or more consecutive pregnancies before the 20th week of pregnancy. If RPL develops following a live birth, it is called secondary, and if there is no history of successful pregnancy, it is called primary RPL. RPL is observed in 1–3% of pregnancy losses (1). The risk is 31% on average in those who have experienced two or more pregnancy losses (2).

Many factors contribute to the etiology of RPL such as parental chromosomal anomalies, fetal anomalies, hypothyroidism, diabetes mellitus, anatomical anomalies of the uterus, antiphospholipid antibody syndrome, endocrine disorders, thrombophilia, immunological abnormalities, infections, and environmental factors. However, the cause of approximately half of RPLs remains unexplained (3).

Chromosomal polymorphisms are considered variations and are defined as differences in the size or staining of chromosome segments (4, 5). Heterochromatic regions are structures that contain tandem repeat sequences and do not encode active genes; they are evaluated as normal karyotype variations, and phenotypic reflections are not expected (6).

This study investigated the relationship between RPL and chromosomal polymorphisms. Cytogenetic analyses were performed on 1688 cases presenting with a history of RPL and a control group consisting of 40 married couples.

MATERIALS and METHODS

Determination of case and control groups

The current study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Decision (Date: 08.09.2017, No: 233). Chromosomal polymorphisms were retrospectively evaluated in 1688 cases, 939 females and 749 males, who visited the Faculty of Medicine in the Department of Medical Genetics at Afyon Kocatepe University, were diagnosed with RPL between 2007 and 2017, and underwent karyotype analysis. Of the 1688 cases evaluated, 665 participated as couples, as some spouses did not provide samples. This retrospective study was conducted on images and archive preparations by selecting archived files from patients who did not have any chromosomal abnormalities or consanguineous marriages. The control group consisted of 40 healthy married couples who had no known hereditary disease, were not relatives, could have healthy children, and had no history of miscarriage and/or stillbirth. Individuals in the control group signed a voluntary consent form, and cytogenetic analyses were performed. Structural and numerical anomalies were excluded from evaluation, and we focused only on chromosomal variants.

Chromosome preparations

From the control group, 2 ml of peripheral blood were collected in heparinized tubes under sterile conditions, and closed lymphocyte culture was performed using standard techniques. The Giemsa-Trypsin-Leischman (GTL) banding technique was applied to metaphase plates. For each individual, 30 metaphase plates at the level of 450-550 bands were obtained. Other banding methods (C banding and NOR banding) were applied when deemed necessary. Three experts examined the metaphase plate using the Applied Imaging Cytovision Image Analysis System. The results were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013.

Statistical analysis

Data were compared in the Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) version 25 software using the $\chi 2$ test, and $p<0.05$ was considered significant.

RESULTS

Of the 1688 cases evaluated retrospectively, 939 were female and 749 were male. There were 665 married couples in this case group. Their ages ranged from 17 to 54 years, and the

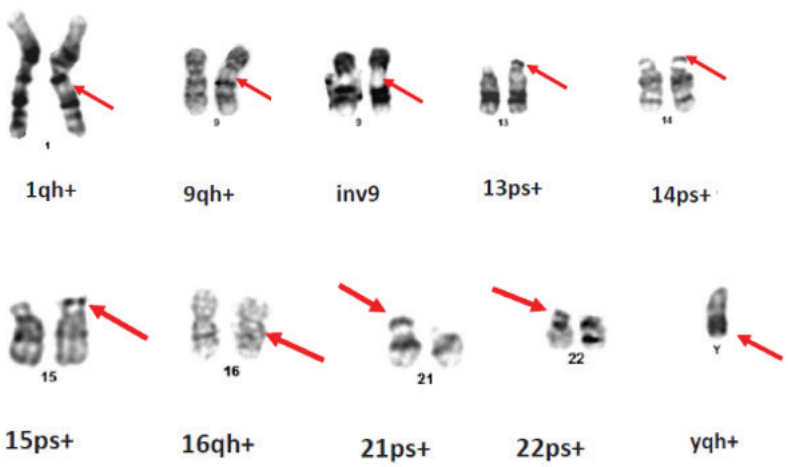

Figure 1. Chromosome images of 10 polymorphic variants detected in the case group

Table 1. The chromosomal polymorphisms and frequencies in the case group (%)

Polymorphism	Female (n=939)	%	Male (n=749)	%
1qh+	3	0.3	8	1.1
9qh+	170	18.1	130	17.2
inv9	11	1.2	7	0.9
13ps+	20	2.1	19	2.5
14ps+	29	3.1	19	2.5
15ps+	24	2.6	15	2.0
16qh+	3	0.3	4	0.5
21 ps+	42	4.5	32	4.3
22 ps+	18	1.9	23	3.1
Yqh+	-	-	1	0.1

Table 2. Distribution of 9qh+ chromosome polymorphisms in the case and control groups

	9qh+		Total	p
	Presence	Absence		
Case group	300	1388	1688	0.028
Control group	5	75	80	
Total	305	1463	1768	

Table 3. Presence of chromosome polymorphism in couples in the case and control groups

	Presence	Absence	Total	p
Case group	305	360	665	0.045
Control group	12	28	40	
Total	317	388	705	

Table 4. Presence of chromosome polymorphisms in individuals in the case and control groups

	Presence	Absence	Total	p
Case group	488	1200	1688	0.014
Control group	13	67	80	
Total	501	1267	1768	

average was 28 years. The ages of the controls, consisting of 40 married couples, ranged from 26 to 70 years, and the average was 41 years.

1qh+, 9qh+, inv9, 13ps+, 14ps+, 15ps+, 16qh+, 21 ps+, 22 ps+, and Yqh+ polymorphisms were observed in the case group (Figure 1). 9qh+ polymorphism was detected at the highest rate (17.2%), whereas Yqh+ polymorphism was detected at the lowest rate (0.1%) (Table 1).

9qh+ polymorphism was detected in 300 out of 1688 individuals in the case group and in five out of 80 individuals in the control group (Table 2). 9qh+ polymorphism was higher in the case group than in the control group ($p<0.05$). (Table 2).

Among couples in the case group, 9qh+ polymorphism was detected in a spouse in 163 couples and in both spouses in 37 couples. In the control group, 9qh+ polymorphism was detected in a spouse of five couples. The distribution differed between the case and control groups ($p<0.05$). 9qh+ polymorphism was significantly higher in the couples in the case group.

Chromosome polymorphisms were detected in 305 of 665 couples in the case group and in 12 of 40 couples in the control group. There was a difference in the presence or absence of polymorphism between the pairs in the case and control groups ($p<0.05$). Polymorphisms were more frequently detected in the case group ($p<0.05$) (Table 3).

Chromosome polymorphism was detected in 488 of 1688 individuals in the case group and in 13 of 80 individuals in the control group. There was a difference between the case and control groups in the presence or absence of polymorphic va-

riants ($p<0.05$). Polymorphisms were observed significantly more frequently in the case group ($p<0.05$) (Table 4). In the retrospectively evaluated case group, the rate of coexistence of two chromosome polymorphisms in an individual was 3.5% (60/1688), whereas the rate of coexistence of three chromosome polymorphisms was 0.29% (5/1688).

DISCUSSION

RPL is a serious health problem that affects couples who want children. Multiple genetic and/or environmental risk factors contribute to the etiology of RL (7, 8), which is observed in 1–5% of pregnancy losses (9-13). Chromosomal anomalies are responsible for 75% of spontaneous early pregnancy losses. More than 90% of fetal chromosome anomalies are numerical anomalies. The remainder consists of structural anomalies and mosaicism (14, 15). Chromosomal polymorphisms are normal variations that occur in 2–5% of the general population; they are usually found in the genetically inactive heterochromatic regions of chromosomes (7). A total of 576 (34.1%) polymorphic variants were detected in 320 female and 256 male individuals out of 1688 cases retrospectively evaluated in the case group. In our study, the 9qh+ polymorphism, which occurred most frequently, was detected in 170 female (18.1%) and 130 male (17.1%) individuals. In the case group, 9qh+ polymorphism was detected in one partner in 163 couples and in both partners in 37 couples. In the control group, the polymorphism was detected in only one individual among five couples. A difference was detected between the case and control groups: 9qh+ polymorphism was frequently observed in the case group couples. In a study conducted in Diyarbakr on 455 couples with RPL, chromosome polymorphism was detected in 8.4% of the case group and 4.9% of the control group. This study, like ours, found an association between RPL and chromosome polymorphisms. However, unlike our study, Akbaş et al. found that the distribution of chromosome polymorphisms in the RPL group was higher in males (11.3%) than in females (5.4%) (16). Other studies suggest that chromosomal polymorphisms contribute to recurrent miscarriages (17, 18). Although our results are compatible with the literature, they also show an accumulation of 9qh+ polymorphisms in Turkey's Afyonkarahisar region.

We did not observe a relationship between the distribution of chromosomal polymorphisms and gender in our study group, which agrees with other studies (18, 19). Sheth et al. reported a significantly higher number of polymorphic variants in women than in men (20), though this may be due to the sex imbalance in the study. However, a study in Mexico found a greater number of chromosomal polymorphisms in men in the RPL group than in women, and the distribution was similar in the control group (17).

Among chromosomal polymorphisms analyzed individually between the case and control groups, variants other than 9qh+ were similarly distributed. However, when evaluated as a percentage, polymorphic variants were increasingly observed in the case group. Though not statistically significant, this may indicate limitations in our control group. Literature data on the deviation of polymorphic variants in RPL cases are variable (21, 22). Karaca et al. found that the frequencies of heteromorphism were similar in 384 case couples and 136 control couples (23). Moreover, several studies found no relationship between heteromorphisms and recurrent pregnancy loss (24-26). On the other hand, Nie and Lu focused on the Y chromosome and found that 16qh+ polymorphisms may cause RPL and infertility (27). In a study conducted with 842 couples with a history of RPL and/or infertility, polymorphic variants in heterochromatin regions were potentially related to RPL (27, 28).

Differences were detected in the distributions of chromosome variations evaluated collectively between the control and case groups. In the case group, the chromosomal polymorphisms were very high. Madon et al. karyotyped 842 individuals admitted for primary infertility or recurrent miscarriages and reported a very high rate of polymorphic variants: 28.82% in males and 17.19% in females. These data support the relationship between chromosomal polymorphisms and RPL. Many studies have shown that polymorphisms, especially in the heterochromatin region, are strongly associated with pregnancy loss (16, 22, 28-32). Polymorphic variants on chromosomes were considered "normal," as heterochromatin has no coding potential, and nucleolar regulatory regions (NOR) containing rRNA-encoding genes are therefore not reflected in the phenotype. However, studies conducted using improved molecular techniques suggest that fertility and viability genes are indeed located in heterochromatin. DNA sequencing of chromosome 9, on which we found qh+ in 17.7% (300/1688) of the case group cases in our study, showed that it is structurally highly polymorphic, is observed with many intra- and interchromosomal duplications, and contains the largest autosomal heterochromatin block (29). Many studies have been conducted using Sanger sequencing to investigate genetic factors in RPL cases. Genome-wide association studies aim to identify genomic regions and SNPs that may be associated with RPL; some polymorphisms in HLA genes, *FHIT*, *FAM154A*, *PDEA*, and *GRIK2* genes may be associated with increased risk, but no significant molecular marker has been identified (33, 34).

CONCLUSION

Our study measured the incidence of RPLs in Afyonkarahisar and genetically analyzed them. Our results highlight the necessity and validity of cytogenetic analyses in couples with RPL. The data herein will guide the couples as they approach subsequent pregnancies. However, our study would benefit from larger case and control groups. More data obtained using molecular cytogenetic techniques, such as FISH and array-CGH, can also guide further research.

Ethics Committee Approval: Ethics committee approval was received for the current study from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Decision (Date: 08.09.2017, No: 233).

Informed Consent: Informed consent was obtained from each patients.

Peer Review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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