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Research Article

COMPARISON OF CHROMOSOME ANALYSIS, FACTOR V LEIDEN, AND PROTHROMBIN G20210A MUTATION RESULTS ACCORDING TO THE NUMBER OF PREGNANCY LOSSES IN RECURRENT PREGNANCY LOSS

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ABSTRACT

Objective: Recurrent pregnancy loss is defined as the loss of two or more pregnancies in some sources, or three or more in others, before 20-24 weeks of gestation. The causes being investigated include parental chromosomal abnormalities and hereditary thrombophilia. We aimed to reveal the frequency of parental chromosome abnormalities, Prothrombin G20210A mutations (PGM), and Factor V Leiden (FVL) in couples presenting with recurrent pregnancy losses and to test whether there is a significant difference between two and more than two pregnancy losses.

Materials and Methods: A total of 171 couples who presented to the Medical Genetics outpatient clinics of two tertiary hospitals located in Bolu and Hatay provinces due to a history of recurrent pregnancy loss were evaluated. Demographic data, medical and family history, chromosomal analysis results of the couples, and FVL and PGM results of the women were recorded.

Results: We detected chromosomal abnormalities in 2.9% of those evaluated. Factor V Leiden frequency was found to be 11.5% and PGM frequency was 3%. No statistically significant distinction was obtained between the groups, categorized as those with two and more than two pregnancy losses, in terms of the occurrence of chromosomal abnormalities (p=0.65), FVL (p=0.58), and PGM (p=0.65).

Conclusion: A similar approach to requesting a test can be taken for both patient groups. Due to the limited number of patients, a meta-analysis of this result with other case series in Turkey would be beneficial.

Keywords: Thrombophilia; Recurrent pregnancy loss; Prothrombin; Factor V; Chromosome abnormalities

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INTRODUCTION

Spontaneous termination of pregnancy between conception and 20-24 weeks of gestation is considered pregnancy loss (1). While some resources define recurrent pregnancy loss (RPL) as miscarriages of two or more pregnancies (1,2), some publications include at least three consecutive pregnancy losses within the scope of this term (3,4). The frequency of this condition is about 1-2% (1,2). The cause is unknown in 50-75% of cases (5). Recurrent pregnancy loss causes increased stress and depression, especially in women (6).

In studies investigating RPL, factors such as genetics, uterine abnormalities, thrombophilia, endocrine disorders, infection, autoimmunity, sperm factors, and personal habits are considered (7). One of the causes of RPL is parental balanced chromosomal abnormalities. The frequency of this situation is 2-4% (8). European Society of Human Reproduction and Embryology (ESHRE) do not routinely recommend chromosomal analysis for parents (9). Whereas American Society for Reproductive Medicine (ASRM) recommends parental karyotyping (2). Royal College of Obstetricians and Gynaecologists (RCOG) recommends chromosomal analysis of abortion material, particularly after a third miscarriage or any miscarriage in the second trimester. Parental karyotyping is recommended if there is no sample or insufficient tissue, or if unbalanced structural chromosomal abnormalities are detected (4). Although different guidelines have contradictory recommendations regarding the indication for parental karyotyping, it is frequently performed in our country.

Another issue where genetic testing is performed regarding RPL is hereditary thrombophilia. Thrombophilia is a condition in which there is a predisposition to venous thromboembolism. It may be inherited or acquired. Inherited thrombophilic disorders encompass deficiency of Protein C, Protein S or antithrombin, FVL, and PGM. Thrombosis in the placental circulation is thought to cause placental insufficiency (10). The substitution of arginine with glutamine at position 506, known as FVL, was initially unearthed by Bertina et al.. This variant interferes with the degradation of Factor V by activated protein C (11). Factor V Leiden mutation heterozygosity enhances the advent of venous thrombosis by 5-fold, whereas homozygosity increases the risk by 50-fold (12). Factor V Leiden allele frequency was high among Europeans at 4.4% (13). The frequency of FVL in healthy Turkish population was found to be 7.9% (345/4276) (14).

Prothrombin G20210A mutation was defined by Poort et al.. This particular variant is situated in the 3' untranslated region of the F2 gene, leading to elevated levels of prothrombin (15). Carriers are 2 to 3 times more prone to the development of thromboembolism (12). The prevalence of PGM in different geographic regions was reported to be 2% (16). In a study carried out in a Turkish population, this rate was 2.3% (17). In the ESHRE guideline, testing for hereditary thrombophilia is not routinely recommended in RPL, but recommended on a research basis or if there are additional risk factors for thrombophilia (9). The RCOG guideline states that FVL and PGM in second trimester abortions can ideally be investigated on a research basis in RPL (4).

The role of PGM and FVL in RPL is controversial (18). There are studies comparing the frequencies of these mutations in individuals with two and more than two pregnancy losses. Different results were found in these studies (19,20). Some publications make comparisons in terms of chromosomal abnormality (20).

We aimed to determine the rate of chromosomal abnormalities, FVL, and PGM in patients who were evaluated for at least two pregnancy losses and to test whether there is a significant difference between two and more than two pregnancy losses.

MATERIALS AND METHODS

Patients

One hundred seventy-one couples who applied to the Medical Genetics outpatient clinics of Bolu Abant Izzet Baysal Training and Research Hospital between February 1, 2020, and July 15, 2022, as well as those who applied to the Hatay Training and Research Medical Genetics outpatient clinics between March 1, 2021, and July 15, 2022, due to experiencing at least two pregnancy losses before 20 weeks of gestation were included. Clinical and laboratory findings of the patients were examined retrospectively through the hospital registry system and files. Demographic data, medical and family history, number of pregnancies and births, total number and the gestational weeks of pregnancy losses, karyotype results of the couples, and FVL and PGM results of the women were recorded. The conduct of this study was attached to the principles of the Declaration of Helsinki, with all participants providing written informed consent. This study provided ethical approval from the Bolu Abant Izzet

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Baysal Training and Research Hospital's Ethics Committee. (Date: 23.08.2022 Decision No: 2022/223).

Chromosome analysis

Peripheral blood samples were collected into sodium heparin tubes. Standard cytogenetic techniques were used in chromosome analysis. After adequate lymphocyte culture, a mitotic inhibitor (colcemid) was added. Application of hypotonic solution followed by fixation was performed. Chromosome slides were prepared, and G banding was applied. At least 20 metaphases were analyzed with a resolution of 500-700 bands. The International System for Human Cytogenetic Nomenclature (ISCN), version 2016, was utilized for reporting.

Thrombophilia mutation test

After DNA isolation was performed from EDTA-whole blood, seven different gene regions were amplified using the Thrombophilia panel kit (Seqline®, Istanbul, Turkey). A fragment analysis-based mutation detection method was employed on a capillary electrophoresis device to simultaneously detect hotspot mutations in FV (Leiden), FII (G20210A), FXIII (V34L), PAI-1 (4G/5G), MTHFR (C677T/A1298C) genes. Only the results for FVL and PGM have been evaluated due to their significance in comparison to other variants reported in the literature (1).

Statistical analysis

All collected data were analyzed with SPSS (IBM, Statistical Package for the Social Sciences) v.22 program. The Kolmogorov-Smirnov test was used to check whether the variables had a normal distribution or not. Frequencies for nominal and ordinal variables and medians with interquartile range for variables that did not follow a normal distribution were utilized. The presence or absence of chromosomal abnormality, FVL, and PGM were independent variables, and the number of pregnancy losses were dependent variables. The participants were categorized into two distinct groups based on their pregnancy history: those who experienced two pregnancy losses and those who had more than two. A comparative analysis was then conducted between these groups, examining the occurrence of chromosomal abnormalities and gene mutations using chi-square and Fisher's exact tests in cross-tables. Statistical significance was assigned by considering a p-value of less than 0.05.

RESULTS

Patients

One hundred forty-five couples were from the province of Bolu, and 26 couples were from the province of Hatay. The median for female ages was 29 (range: 28 minimum: 18 maximum: 46), and the median for men ages was 32 (range: 31 minimum: 18 maximum: 49). The median number of pregnancy losses was 2 (range:4 minimum:2 maximum:6). 57% of the couples had two pregnancy losses (n=97), 34.5% had three pregnancy losses (n=59), 4.7% had four pregnancy losses (n=8), 3.5% had five pregnancy losses (n=6), 0.6% had six pregnancy losses (n=1). 35.1% of the patients had a history of live birth, and 64.9% did not (Table 1).

Table 1. Clinical and laboratory characteristics of the patients, N=171

Age	
Female	Median 29 (Range: 28, Min: 18, Max: 46)
Male	Median 32 (Range: 31, Min: 18, Max: 49).
Number of pregnancy loss	
2	N=97 (57%)
3	N=59 (34.5%)
4	N=8 (4.7%)
5	N=6 (3.5%)
6	N=1 (0.6%)
Healthy offspring	
Yes	N=60 (35.1%)
No	N=111 (64.9%)
Chromosome abnormality	
Female	N=3 (1.75%)
Male	N=2 (1.17%)
Factor V Leiden	
Wild	N=147 (88.5%)
Heterozygous	N=19 (11.5%)
Homozygous	N=0
Prothrombin G20210A	
Wild	N=161 (97%)
Heterozygous	N=5 (3.0%)
Homozygous	N=0

Chromosome analysis

Chromosome abnormalities were detected in two women and three men out of 171 couples. There was a chromosomal abnormality in 2.9% of the couples. Chromosomal abnormalities are shown in Table 2. Reciprocal translocations were detected in four cases, and sex chromosome abnormality was detected in one case. Upon categorizing the cases into two groups based on the number of pregnancy losses, chromosomal abnormalities were detected at similar frequencies in both groups (n=2, 2.1% in two pregnancy losses; n=3, 4.1% in more than two pregnancy losses, p=0.65).

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Table 2 Parental chromosome abnormalities

Chromosome Abnormality	Number of patients (n)
46,XX t(1;7)(p36.2;p21)	1
46,XX,t(11;22)(q23;q11.2)	1
46,XX,t(12;22)(q22;q11.2)	1
46,XY,t(9;11)(q33;p15)	1
mos 47,XYY[15]/46,XY[35]	1

Factor V Leiden and Prothrombin G20210A Mutation

Mutation analyses of 166 patients were evaluated. We excluded those with abnormal chromosome results. Factor V Leiden frequency was 11.5% (19/166), and PGM frequency was 3% (5/166) (Table 1). No homozygous or double heterozygous patients were detected. When compared between the two abovementioned groups, similar rates were detected for both FVL and PGM. (FVL (p=0.58); PGM (p=0.65)) (Table 3 and 4). No significant difference was detected between mutation carriers and noncarriers in the history of live birth. Whereas the live birth history rate was 32.9% (48/146) in noncarriers, 46.2% (12/26) of the mutation carriers had a live birth history (p=0.19).

Table 3. Comparative analysis of Factor V Leiden mutation rate in cases with two pregnancy losses versus three or more pregnancy losses

Factor V G1691A	Two pregnancy losses (n=95)	Three or more pregnancy losses (n=71)
GG /Wild	83 (87.4%)	64 (90.1%)
GA/Heterozygous	12 (12.6%)	7 (9.9%)
AA/Homozygous	0	0

Chi-square; p=0.58

Table 4. Comparative analysis of Prothrombin G20210A mutation rate in cases with two pregnancy losses versus three or more pregnancy losses

Prothrombin G20210A	Two pregnancy losses (n=95)	Three or more pregnancy losses (n=71)
GG /Wild	93 (97.9%)	68 (95.8%)
GA/ Heterozygous	2 (2.1%)	3 (4.2%)
AA/ Homozygous	0	0

Fisher's exact test; p=0.65

DISCUSSION

Couples experiencing recurrent pregnancy loss are apprehensive about having another pregnancy loss. They want the underlying cause to be identified and the situation resolved as soon as possible. As geneticists, we carry out chromosome analysis and hereditary thrombophilia testing as part of our daily practice. When we evaluated the test results of 171 couples, we detected a chromosomal abnormality in 2.9% (5/171) of them. While balanced reciprocal translocations were detected in four cases, sex chromosome abnormalities were detected in one. The most frequently detected chromosomal abnormality is reciprocal translocation, which is consistent with the literature. The sex chromosome abnormality we detected has rarely been reported in RPL in the literature (21). The patient may be prone to meiotic nondisjunction (22). If a parental balanced structural change is detected, preimplantation genetic testing and invasive prenatal procedures should be recommended for future pregnancies. The chances of a healthy pregnancy are influenced by the gene regions involved and the type of rearrangement (7). Genetic counseling is crucial for these couples. Detected chromosomal translocation abnormalities should also be screened for other family members. It can be helpful not only for the couple but also for the other potential carrier relatives. Additionally, chromosome analysis and microarray investigations on abort materials will be very informative in some cases. Microarray analysis does not need viable cells, so they could be preferred when it is possible.

Additionally, participants were categorized as having two or more pregnancy losses. Indistinguishable results were detected in two groups in terms of the frequency of chromosomal abnormality (n=2, 2.1% two pregnancy losses; n=3, 4.1% more than two pregnancy losses; p=1). Youssef et al. found no difference between the groups in the prevalence of chromosomal abnormalities in a study including 240 patients (23). Our result was in agreement with a meta-analysis (10 studies, OR 0.78, 95% CI 0.55–1.10). The rate of chromosomal abnormality was found to be 5.3% in two pregnancy losses, whereas it was 6.6% in more than two pregnancy losses (20). Although the number of cases is limited in our study, a similar result was found.

We evaluated hereditary thrombophilia risk factors in women. We found the frequencies of FVL and PGM in our patients to be 11.5% and 3%, respectively. Different RPL studies conducted in our country found varying rates, such as 7.9%, 9.5%, 10%, 11.2%, and 16.9% for FVL. The rates for PGM were 1.7%, 2.1%, 3.5%, 5.4% and 14.1% (24-27,19). In two studies conducted in control groups, the frequency of FVL was 7% and 11%, and the frequency of PGM was 1.6% and 5% (24,27). The effects of hereditary thrombophilia in the etiology of RPL are controversial. Although some studies reveal that FVL and PGM are associated with it (28,29,30), others do not support this argument (31,32,33). Also, two studies from our country that compare RPL and control patients did not support this

relationship (24,27). Factor V Leiden and PGM continue to be investigated in the etiology of RPL. The pregnant women detected to have thrombophilia are using anticoagulant drugs for a healthy ongoing pregnancy. These drugs have some adverse effects, like a tendency to bleed, bruising at the application site, and pain. The confusion over whether thrombophilia is a contributing factor to recurrent pregnancy loss (RPL) requires clarification. A study was recently published reporting that Low Molecular Weight Heparin (LMWH) treatment did not lead to an improvement in the live birth rate. In this study, pregnancy success in cases with FVL was found to be 70.8% (68/96) in the group using anticoagulants and 69.9% (58/83) in the group not using drugs. In prothrombin gene mutation, these rates were found to be 72.3% (26/36) and 73.2% (30/41), respectively (34). We compared the status of the healthy live birth history of the patients with thrombophilia mutation carriers and noncarriers. We did not obtain any significant difference. Whereas the live birth history rate was 32.9% (48/146) in noncarriers, 46.2% (12/26) of the mutation carriers had a live birth history (p=0.19). Although we do not know the patient's other risk factors for hypercoagulation, this result may also be related to the controversial effects of thrombophilia genetic factors in RPL etiology.

There are studies comparing the frequencies of these mutations in patient groups with two and more than two pregnancy losses. We detected no remarkable difference between the groups regarding thrombophilia mutations (FVL p=0.58; PGM p=0.65). In contrast to our results, one study conducted with 2660 people from Turkey found that heterozygosity for FVL and PGM was more common in three or more pregnancy losses (p<0.01) (19). The findings of Karadeniz et al. were comparable to ours (35). In a study including 75 cases from Turkey, Kovalak et al. detected a higher FVL mutation rate in three pregnancy loss group compared to two (p=0.029). They did not find any significant difference for PGM. The number of patients are very limited in that study. The chromosome results are not convenient to evaluate since some polymophisms were evaluated as abnormalities (36). Our results were compatible with a meta-analysis (FVL (five studies, n=1109 OR 0.79, 95% CI 0.43-1.47) and PGM (five studies, n=1330 OR 1.08, 95% CI 0.44) -2.62)) (20).

In this study, the results of the chromosomal analysis revealed no significant differences between the comparison groups. In the first group, consisting of two pregnancy losses (n=2), the rate was 2.1%. In contrast, the second group, which experienced more than two pregnancy losses (n=3), showed a rate of 4.1%. No statistical significance was found (p=1). Furthermore, the examination of thrombophilia mutations also showed no remarkable differences between the groups. Factor V Leiden mutations were found in 12 individuals (12.6%) in the two pregnancy loss group, and it was found in 7 individuals (9.9%) in the three pregnancy loss group. No statistically significant difference was detected (p=0.58). Similarly, PGM were present in 2 individuals (2.1%) in the two pregnancy loss group and 3 individuals (4.2%) in the three pregnancy loss group, with a p-value of 0.65, indicating a lack of significant difference.

While there is controversy between our thrombophilia results and several other studies from Turkey, our findings related to chromosomes and thrombophilia were consistent with a meta-analysis (20). We suggest that a similar approach of testing algortihm could be applied to both groups. However, further research is necessary to draw definitive conclusions.

This study has several limitations. First of all, it is really difficult to divide these patients into different groups because, of course, there is a possibility that some women in the first group (with two pregnancy losses) will move to the second group (with more than two pregnancy losses) in the following years. Secondly, because the mutation frequencies are low, our sample can be considered a relatively small sample. The number of patients attending the clinic due to the effects of the Covid-19 pandemic is below expectations. However, we hope the presentation of 2.5 years of patient data will contribute to the literature. Finally, in our study, we included all couples who presented to our clinic and experienced a pregnancy loss before 20 weeks gestation, with no exclusion criteria. For this reason, there may be couples in the sample whose pregnancy loss is due to non-genetic factors. However, given that the most common reason for referral to our clinic is that no other cause of pregnancy loss can be found and that genetic testing is often not requested when a known cause is present, this likelihood is not very high.

CONCLUSION

How many pregnancy losses should be considered as recurrent pregnancy loss is still a matter of debate. No statistically significant distinction was obtained between the groups, categorized as those with two and more than two pregnancy losses, in terms of the occurrence of chromosomal abnormalities, FVL, and PGM. A similar approach of testing algortihm can be applied for both groups. However, due to the limited number of patients, a meta-analysis combining our study with other case series in the Turkish population would be beneficial. In order to

gain a deeper understanding of the etiopathological connection of these genetic changes within the Turkish population, additional functional studies are needed.

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Authorship contributions
Surgical and Medical Pactices: HÖ, MK Concept: HÖ, MK Design: HÖ, MK Data collection or processing: HÖ, MK Analysis-Interpretation: HÖ, MK Literature search: HÖ Writing: HÖ

Data availibity statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest
The authors declare that they have no conflict of interest.

Ethics
This study provided ethical approval from the Bolu Abant Izzet Baysal Training and Research Hospital's Ethics Committee. (Date: 23.08.2022 Decision No: 2022/223).

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