http://www.cjmb.org

Open Access Brief Report

Crescent Journal of Medical and Biological Sciences
Vol. 7, No. 1, January 2020, 134–137
eISSN 2148-9696

Association Study of Recurrent Abortion With Chromosomal Abnormalities and Mutation of Prothrombin Gene in 100 Affected Women in the Northwest of Iran

Seyed Ali Rahmani[1*], Reihaneh Amiri[2], Parisa Mosapour[3]

Abstract

Objectives: Recurrent miscarriage is defined as two or more abortions happening consecutively within less than the twenty weeks of gestation or when the fetus's weight is below 500 g. Numerous factors are involved in recurrent miscarriages, the most important of which are chromosomal abnormalities and females' genetic clotting condition, thrombophilia, including Factor II, prothrombin, and mutation. The present study aimed at investigating the relationship between the couples' chromosomal abnormalities and prothrombin mutation in women with recurrent miscarriages in the northwest of Iran.

Materials and Methods: In the present applied research, 100 couples referring for recurrent miscarriages were subjected to cytogenetic experiments via using the GTG banding. Deep vein thrombosis tests were also conducted on the women based on the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method.

Results: Ten out of 100 studied couples were diagnosed with chromosomal abnormalities. All these abnormalities were of structural type. Out of 100 women subjected to clotting factor II, only one heterozygous case was found while the remaining cases were healthy. The control group subjects (n=100) were also found healthy. No significant difference was evidenced between the control and patient groups.

Conclusions: Based on the results, 5% of the studied cases had structural chromosome abnormalities and this was in compliance with the results obtained in the prior research. As regards the prothrombin mutation, only one out of one hundred studied women was heterozygous whereas the remaining subjects were healthy; this is consistent with the results obtained in previous studies.

Keywords: Chromosomal anomalies, Recurrent miscarriage, Prothrombin gene

Introduction

Recurrent miscarriage is realized as two or more abortions (1) occurring consecutively within twenty weeks of pregnancy or when the fetus is below 500 g in weight (2,3). In addition, it is a condition entangling 2% to 5% of the couples (3,4). It can be a consequence of thrombotic, fibrinolytic, genetic, infectious, chromosomal, anatomic, endocrinal or immune abnormalities, the anatomical anomalies of the uterus, and environmental factors (5). However, 50% of the recurrent miscarriage cases occur for no known reason (6). Chromosomal abnormalities, as the most common cause of the early sporadic abortions, are also reported in the majority of the studies as the contributing factor to a large volume of recurrent miscarriage cases (7,8).

Several studies conducted on the thrombophilic gene mutations have pinpointed that the mother's congenital thrombophilia is related to recurrent miscarriage (9-11). The influential factors in congenital thrombophilia include a mutation in factor V Leiden, protein C deficiency, antithrombin deficiency, and mutation in methylenetetrahydrofolate reductase, PAI1, and prothrombin (12).

Considering the above-mentioned discussions, the present study investigated the relationship between couples' chromosomal abnormalities and recurrent miscarriage, as well as the relationship between the hereditary thrombophilia factors and the prothrombin gene (G20210A) with recurrent miscarriage in female patients.

Materials and Methods

The present applied research examined the chromosomal abnormalities of 100 couples (200 individuals) with two

Received 12 December 2018, **Accepted** 1 April 2019, **Available online** 22 April 2019

[1]Department of Medical Genetics,Tabriz University of Medical Sciences, Tabriz, Iran. [2]Department of Molecular Genetics, Science and Research Branch, Islamic Azad University, Tabriz, Iran. [3]Department of Obstetrics and Gynaecology, Tabriz University of Medical Sciences, Tabriz, Iran.
***Corresponding Author:** Seyed Ali Rahmani, Tel: +989121961050, Email: rahmaniseyedali@yahoo.com

or more abortions during 2015-2017 in Dr. Rahmani's Medical Genetic Laboratory. To this end, the simple random sampling technique was used and sampling was determined by the amount that could be collected in one year. The average ages of the women and the men were 28 and 32 and the number of abortions ranged between 2 and 8.

Standard Cytogenetic Analysis

Peripheral blood (5 mL for every individual) was collected using syringes containing heparin. Between 10 and 12 blood droplets were grown for every individual on a culture medium containing 5 mL RPMI1640 and the GTG banding of the metaphasic chromosomes was performed based on standard methods.

Polymerase Chain Reaction Method

The gene mutation of G20210A prothrombin was examined based on restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). Further, the HindIII enzyme was used for the analysis of the prothrombin G20210A gene mutation. The sequence of the primers is presented in Table 1.

The purity percentage of the extracted DNA was investigated using the Nanodrop device following which the fragments were observed in the buffer loading under the UV light. The results of the PCR section were examined by Fisher exact and chi-square tests.

Results

Cytogenetic Part

All 100 couples were subjected to the experiments. The female and male patients' average ages were 28 (in a range of 17-45 years) and 32 (within the range of 22-59 years), respectively. All the 100 couples were subjected to chromosomal analyses. Ten out of 200 analyzed subjects were diagnosed with chromosomal disorders (5% of cases and 10% of couples). Furthermore, women and men accounted for 6 (60%) and 4 (40%) cases of abnormalities (Table 2).

No couples were found with chromosomal abnormalities in both male and female partners.

Molecular Part

To study the relationship between prothrombin gene mutation and recurrent miscarriage, 100 women with recurrent miscarriages and 100 normal women (as the control group) were investigated, who were within the age range of 17-45 and 19-42, respectively.

No mutation was found in the control group and only one heterozygous mutation was observed in the patient group. Chi-square and Fisher exact tests were employed to compare the patient and control groups (Table 3).

The level of Fisher's weight is 1. Given that this value is greater than 0.05, it can be indicated that the mutation level fails to differ significantly between the control and patient groups. Thus, it was concluded that prothrombin gene mutation is rare in northwest Iran, implying that it is not clinically worthy of diagnosis.

Controlling factors for preventing recurrent abortions:

1. Proper nutrition;
2. Precise use of prescription drugs by the doctor;
3. Enough rest;
4. Lack of stress and worry.

Frequent abortion labor constraints:

1. The cost of testing and treatment of recurrent abortions;
2. The right time for taking action to diagnose the disease.

Suggestions for Future Studies in this Field

1. Comparing the rare chromosomal polymorphisms such as chromosome 1 in normal populations and the population with recurrent abortion in order to decide appropriately and better evaluate the association between this polymorphism and recurrent abortion, considering that our control sample had at least two healthy children and had no history of abortion.
2. Investigating rare chromosomal polymorphisms in samples with a greater number to obtain better and more accurate results.

Table 1. Sequence of Primers

Forward primer	5'GCACAGACGGCTGTTCTCTT 3'
Reverse primer	3' ATAGCACTGGGAGCATTG 5'

Table 2. Spectrum of Chromosomal Abnormalities Detected From the Chromosome Analysis of 100 Couples Performed for the Investigation of Recurrent Etiology

Percent in 100 Couples (200 Individuals)	Structural Abnormalities (n=10)	No. of Cases
0.5%	45xx, rob (14:21)	1
0.5%	46xy, inver (9)	1
0.5%	46xy, $1qh^{+}$	1
0.5%	46xy, $21ps^{+}$	1
0.5%	46xx, $14ps^{+}$	1
1%	46xx, $15ps^{+}$	2
0.5%	46xy, $22ps^{-}$	1
0.5%	46xx, $22ps^{-}$	1
0.5%	46xy, $15p^{+}$	1

Table 3. Cross-tabulation Gene Mutation and Group

Group	Mutant Gene		Total
	Positive	Negative	
Patient	100 (100%)	99 (99%)	1 (1%)
Control	100 (100%)	100 (100%)	0 (0%)
Total	200 (100%)	199 (99.5%)	1 (0.5%)

3. Studying the amount of satellite extension in acrocentric chromosomes and the rate of chromosome nine reversal in normal populations and those with recurrent abortion in order to better understand these abnormalities regarding the control groups with at least two healthy children and no history of abortion.
4. Determining the amount of satellite extension in acrocentric chromosomes and the rate of the reversal of chromosome number nine in further specimens for more accurate verification of these reversals with recurrent abortion.
5. Investigating the relationship between prothrombin mutation and recurrent abortion in different societies in order to better understand the effect of this mutation in recurrent abortions in other societies.

Discussion

Chromosomal abnormalities were observed in 2% to 8% of the couples referring for recurrent miscarriage. The present study found chromosomal abnormalities in 5% of all the cases and 10% of couples. Based on previous studies, the couples' chromosomal abnormalities are responsible for recurrent miscarriages. The comparison between the results of previous studies and those of the present study is provided in Table 4.

All the conducted studies demonstrated the accentuated role of the couples' chromosomal abnormalities in recurrent miscarriage. As shown, the obtained results in the current research are in line with those of the other studies and the percentage differences are due to the differences in the size and type of the study sample volume.

Thrombophilic abnormalities are the underlying cause of placental perfusion anomalies because the venous vein and intervillous thrombosis bring about late pregnancy conditions such as late abortion and preeclampsia (13). The current study evaluated 100 women with recurrent miscarriage and 100 healthy women (as the control subjects) in molecular terms based on the RFLP-PCR method. According to the obtained results, out of the 100 women with recurrent miscarriage, only one 33-year-old woman with two abortions was found heterozygous with respect to prothrombin gene mutation and the other women with recurrent miscarriage were normal with regard to the gene. No mutation was documented for the control group as well. The results of various studies are described as follows.

Souza et al (17) studied the prothrombin gene mutation in 56 women with recurrent miscarriage. Based on the results, two cases of heterozygous genes (3%) were found among these patients in terms of prothrombin gene mutation. Four women out of 384 healthy control group women had heterozygous genes with regard to prothrombin mutation. However, no significant difference was observed between the mutation rates of the control and patient groups.

Similarly, Di Micco et al (18) studied the women with recurrent miscarriage and concluded that prothrombin gene mutation rarely happens in heterozygous and homozygous forms in women with recurrent miscarriage, which is in line with the results of the present study regarding the heterozygous form. Finally, Poursadegh Zonouzi et al (19) studied 89 women with recurrent miscarriage and 50 healthy women as the control group and concluded that 1.12% and 1.12% of prothrombin gene mutation in women with recurrent miscarriage were heterozygous and homozygous, respectively. No mutation was observed in the control group. In addition, no significant difference was detected between the control and patient groups in terms of gene mutation.

Conclusions

The present study evaluated chromosomal abnormalities in women diagnosed with recurrent miscarriage and the obtained results were consistent with the findings of prior research. Couples' chromosomal abnormalities play a direct role in recurrent miscarriage which happens even in the case that one of the couples is diagnosed with chromosomal abnormalities. As for the prothrombin gene mutation, only one heterozygote case (1%) was found in the patient group while no case of mutation was evidenced in the control group. Considering the results of the present study and those of previous studies, the prothrombin gene mutation is rare at least in the northwest of Iran based on the absence of a significant difference between the patient and control groups in terms of prothrombin mutation. Therefore, it is clinically worthless of detection and not recommended for the women who refer for recurrent miscarriage in the northwest of Iran.

Table 4. Comparison of the Results of Previous Studies With Those of the Present Study

Study	Year	Ethnicity	Case (Couple)	Karyotype Anomaly (%)	Chromosomal Anomaly (%)	Polymorphic Variant (%)
Our study	2017	Northwest of Iran	100	5	1.5	3.5
Lucas et al (13)	1972	London	42	3.57	3.57	0
Dubey et al (14)	2005	India	742	2	0.58	1.41
Ocak et al (15)	2013	Turkey	495	2.8	2.3	0.5
Gonçalves et al (16)	2014	Brazil	94	7.3	1.45	5.85

Conflict of Interests

Authors have no conflict of interests.

Financial Support

None.

References

1. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368(9535):601-611.
2. Roman E. Fetal loss rates and their relation to pregnancy order. J Epidemiol Community Health. 1984;38(1):29-35. doi:10.1136/jech.38.1.29
3. Kuo PL, Guo HR. Mechanism of recurrent spontaneous abortions in women with mosaicism of X-chromosome aneuploidies. Fertil Steril. 2004;82(6):1594-1601. doi:10.1016/j.fertnstert.2004.06.042
4. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril. 2001;75(4):678-682. doi:10.1016/s0015-0282(00)01801-x
5. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fertil Steril. 2010;93(4):1234-1243. doi:10.1016/j.fertnstert.2009.01.166
6. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. Curr Opin Endocrinol Diabetes Obes. 2009;16(6):446-450. doi:10.1097/MED.0b013e3283327fc5
7. Sierra S, Stephenson M. Genetics of recurrent pregnancy loss. Semin Reprod Med. 2006;24(1):17-24. doi:10.1055/s-2006-931797
8. Toncheva D. Fragile sites and spontaneous abortions. Genet Couns. 1991;2(4):205-210.
9. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol. 2006;55(5):360-368. doi:10.1111/j.1600-0897.2006.00376.x
10. Goodman CS, Coulam CB, Jeyendran RS, Acosta VA, Roussev R. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? Am J Reprod Immunol. 2006;56(4):230-236. doi:10.1111/j.1600-0897.2006.00419.x
11. Yenicesu GI, Cetin M, Ozdemir O, et al. A prospective case-control study analyzes 12 thrombophilic gene mutations in Turkish couples with recurrent pregnancy loss. Am J Reprod Immunol. 2010;63(2):126-136. doi:10.1111/j.1600-0897.2009.00770.x
12. Moghaddasi MH, Samiee S, Amini Kafi Abad S, Attaee Z, kavari M, Sobhani M. The prevalence of G20210A gene mutationas in thrombotic patients. The Scientific Journal of Iranian Blood Transfusion Organization. 2008;5(2):67-72. [Persian].
13. Lucas M, Wallace I, Hirschhorn K. Recurrent abortions and chromosome abnormalities. J Obstet Gynaecol Br Commonw. 1972;79(12):1119-1127. doi:10.1111/j.1471-0528.1972.tb11898.x
14. Dubey S, Chowdhury MR, Prahlad B, al. Cytogenetic causes for recurrent spontaneous abortions–an experience of 742 couples (1484 cases). Indian J Hum Genet. 2005;11(2):94-98.
15. Ocak Z, Özlü T, Ozyurt O. Association of recurrent pregnancy loss with chromosomal abnormalities and hereditary thrombophilias. Afr Health Sci. 2013;13(2):447-452. doi:10.4314/ahs.v13i2.35
16. Gonçalves RO, Santos WV, Sarno M, Cerqueira BA, Gonçalves MS, Costa OL. Chromosomal abnormalities in couples with recurrent first trimester abortions. Rev Bras Ginecol Obstet. 2014;36(3):113-117. doi:10.1590/s0100-72032014000300004
17. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF. Factor V leiden and factor II G20210A mutations in patients with recurrent abortion. Hum Reprod. 1999;14(10):2448-2450. doi:10.1093/humrep/14.10.2448
18. Di Micco P, D'Uva M, Strina I, et al. Recurrent pregnancy loss and thrombophilia. Clin Lab. 2007;53(5-6):309-314.
19. Poursadegh Zonouzi A, Chaparzadeh N, Ghorbian S, et al. The association between thrombophilic gene mutations and recurrent pregnancy loss. J Assist Reprod Genet. 2013;30(10):1353-1359. doi:10.1007/s10815-013-0071-5