

Measurement of TGFB1 and JAK2 Polymorphism in the Blood Cells of Iranian women with Recurrent miscarriage

Saeed Zaker Bostanabad^{1,*}, Soheila Darmanloo², and Somayeh Darmanloo³

¹ Scientific-Applied Training Center of Farzanegan Andishmand Massoud (FAM), Tehran, Iran

² Shafa neuroscience research center, khatam Alanbia Hospital, Tehran, Iran

³ Department of Biology, Islamic Azad University, Parand Branch, Tehran, Iran

*Corresponding author: Saeed Zaker Bostanabad, Ph.D. saeedzaker20@yahoo.com

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Abstract

Introduction and aim: Recurrent miscarriage is two or more consecutive pregnancy losses before weeks 20. Different factors involved in recurrent abortion as a multifactorial disease. Genetic factors are one of the important factors associated with recurrent abortion. The goal of this article is to assess the association between single nucleotide polymorphisms of TGFB1 and JAK2 polymorphism and the recurrent miscarriage in Iranian women.

Methods: The study group was 30 women with recurrent miscarriages with unknown reason and the control groups consisted of 10 women with at least two successful pregnancies and no miscarriages. PCR was used to find the association between TGFB1 and JAK2 genes and recurrent miscarriages. Analysing performed with FinchTV software after data sequencing.

Results: Our results provided evidence that TGFB1 polymorphisms influence the risk of recurrent abortion in the Iranian population however there is no correlation with JAK2 polymorphism and recurrent abortion.

Conclusion: This finding could help to prevent recurrent miscarriage in Iranian women.

Keyword: Blood Cells; Polymorphism; TGFB1; JAK2; Recurrent abortion

Introduction

Polymorphism analysis of the genes involved in thrombophilia should be considered in several cases, and the discovery of the association of this polymorphism with recurrent miscarriage may offer better treatment options. The most common complication in the first and second trimesters of pregnancy is abortion. The

concept of abortion refers to the termination of pregnancy before the 20th week. Among women who experience more than two consecutive abortions, they have recurrent miscarriages (Aruna *et al.*, 2010). Spontaneous abortion is not uncommon and about 15% of clinically diagnosed pregnancies lead to spontaneous abortion (Rai and Regan, 2006). This complication happens in 5 to 50% of

pregnancies (Salat-Baroux, 1988). Considering that fertility is highly valued in most cultures and the desire to have a child is one of the most basic human standards. If pregnancy attempts fail, it can lead to a devastating feeling and a stressful event that can lead to mental health problems (Bjørnerem *et al.*, 1997). It is one of the most debilitating illnesses in women, leading to physical, mental, and economic costs. (Rai and Regan, 2006). In many studies conducted in different countries, different results have been obtained, in women who had an abortion increases the risk of mental health problems after abortion the psychiatric problems, including Depression (anxiety - sometimes severe emotional distress after abortion and suicide attempt - substance abuse - obsessive-compulsive disorder) and marital arguments (Hutti *et al.*, 2011). Also, quality of life and physical-emotional and social performance in women with a history of abortion are lower than in the normal population (Nansel *et al.*, 2005). Pregnancy is a clot secondary status. Because of an increase in coagulation factors, a decrease in the natural occurrence of anticoagulants, and a fibro lysis disorder occur (Daya *et al.*, 1998). There is probably an adaptive physiological mechanism to prevent postpartum hemorrhage, however, when combined with another clot-like condition such as intrinsic thrombophilia, it can lead to thrombosis line 1 (Pabinger *et al.*, 2009). Increased clotting in pregnancy, especially due to hereditary thrombophilia, can lead to placental vascular thrombosis (Kupferminc, 2003). Fetal health is directly related to the maternal circulation, and anything that impairs this connection, it is harmful to the fetus. It seems that malignant clots or thrombosis in placental capillaries can interfere with the process of material exchange between the mother and the fetus and eventually lead to miscarriage (Kupferminc, 2003). About 55% of patients have a recurrent miscarriage of thrombophilia (Kupferminc, 2003). Thrombophilia presents as venous thromboembolism, deep vein thrombosis, pulmonary embolism, myocardial infarction,

and cerebral vein thrombosis (Kempf and Klimek, 2005). Inappropriate clot thrombosis can, to the extent of placental capillaries, disrupt the process of material exchange between the mother and the fetus and eventually lead to miscarriage (Gnanasambandan *et al.*, 2010).

The TGFB1 gene offers instructions for generating a protein called transforming growth factor beta-1 (TGF β -1) which is a multifunctional cytokine with pro-inflammatory effects in some settings and anti-inflammatory effects in others. TGF- β 1 is produced by T regulatory lymphocytes (Treg), which perform an essential role in the physiology of pregnancy. The TGF-B1 gene is located on chromosome 19 and contains 7 exons. Numerous single nucleotide polymorphisms (SNPs) have been described in the TGF-B1 gene at positions -988, -800, and -509 located in the gene and have been analyzed for connection with a distinct disease (Amani *et al.*, 2005).

The Janus kinase-2 (JAK2) gene offers instructions for making a protein that promotes the growth and division (proliferation) of cells and this gene placed on the short arm of chromosome 9 (9p24), encodes a protein that promotes the growth and cell proliferation. JAK2 is a cytoplasmic tyrosine kinase protein, which facilitates signal-transducing downstream of several cytokine receptors implicated in erythropoietin receptor signaling and hematopoiesis. JAK2 is a member of the Janus kinase family and has been involved in signaling via members of the single-chain receptors, the type II cytokine receptor family, the gp130 receptor family, and the GM-CSF receptor family (IL-3R, IL-5R, and GM-CSF-R). The JAK2V617F mutation has been detected in women with unexplained recurrent miscarriage.

Repeated mutations of the JAK2 gene V617F cause hematopoietic cells to grow in the bone marrow and cause venous thromboembolism. It is a serious indicator of myeloproliferative diseases and as a result of venous thrombosis. Therefore, the molecular identification and

structure of the V617F mutation of the JAK2 gene are important for the treatment and understanding of clinical value (Gnanasambandan *et al.*, 2010). Mutations in the JAK2 gene have been shown to increase cytokine sensitivity and induce erythrocytosis in the mouse model (Lacout *et al.*, 2006).

According to the prevalence of miscarriage in women of about 5%, the devastating psychological effects of abortion on family life and the cause of some of these abortions are coagulation problems, in this study, we evaluated the association between JAK2 polymorphism and its association with spontaneous abortion in Iranian patients and healthy controls group.

Methods

Patients and samples: This cross-sectional study included 40 women dividing into two groups. The first group is 30 women had a history of recurrent abortion and the 10 control women who had 2 successful pregnancy with no history of recurrent abortion. All the women who attended Massoud clinical laboratory in 2019. Blood samples were taken from all of the women (5 ml from each woman). Non-spontaneous abortion due to chromosomal abnormalities in the fetus, anatomical problems in the uterus, and age-related abortion infections and hypothyroidism were considered as exclusion criteria.

DNA extraction: After blood sampling, the blood was poured into EDTA-containing tubes and DNA extracted utilizing the Sina Clone Star DNA purification kit, following manufacturer's protocols. The amount of DNA extracted was then measured by Nanodrop (Hekmat *et al.*, 2020).

Primer design: The TGFB1 and JAK2 polymorphisms were detected by primers R and F for each of the two polymorphisms. To design the required primers, the region of the target gene was first sequenced from the NCBI site (<http://www.ncbi.nlm.nih.gov/>) and afterward, the appropriate primers were designed by the

NCBI Primer-blast tool. Subsequently, the structure of each primer designed to ensure their specificity was examined by gene runner software version 6.0.28. The designed primers are described in Table 1.

Table 1 Features of designed primers

| Genes | Primer sequence (5' to 3') |
|--------------|------------------------------|
| TGFB1 | |
| Forward | TGTTTCGCGCTCTCGGCAGTG |
| Reverse | AGCCAGTTTCTTCTGCCAGT |
| JAK2 | |
| Forward | CAAAGTTCAATGAGTTGACC CCTA |
| Reverse | CTTATGAAAAATATGCCAAC CTTG |

PCR analysis: The PCR reaction was done in 25 µl volume: 9 µl Master mix, 1 µl specific primers, 2 µl DNA and 13 µl distilled water. The amplification conditions were as follows: one cycle of 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 10 s, 30 s at 58 °C and 20 s at 72 °C. After PCR, the genes were accomplished as follows: from 56 °C to 99 °C, the temperature was increased by 0.1 °C/2 s. The PCR product was then electrophoresed on 1% agarose gel.

Data analysis: All PCR-amplified DNA was then sent to Royan Biogen Gene for sequencing. After the sequence, all data were analyzed with FinchTV software. The data were analyzed by SPSS 16 using the Chi-squared test and Fisher exact tests. The significance level of tests was considered less than 0.05.

Results

Figures 1 and 2 show the agarose gel electrophoresis of TGFB1 PCR for the control group (women had 2 successful pregnancy with no history of recurrent abortion), and experimental group (women had a history of recurrent abortion), respectively.

Figures 3 and 4 show the agarose gel electrophoresis of JAK2 PCR for control group and experimental group (women had a history of recurrent abortion), respectively.

The data then were analyzed with the Chi-square test and the results were shown in Table 2 and Table 3.

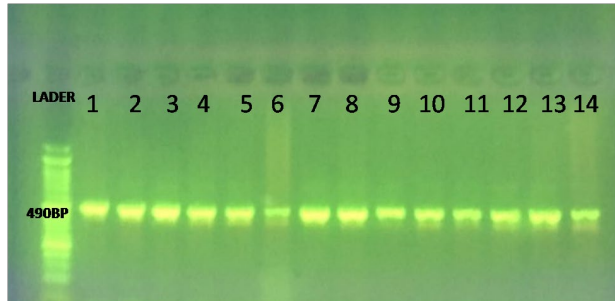


Figure 1. Agarose gel electrophoresis of TGFB1 PCR of control group samples.

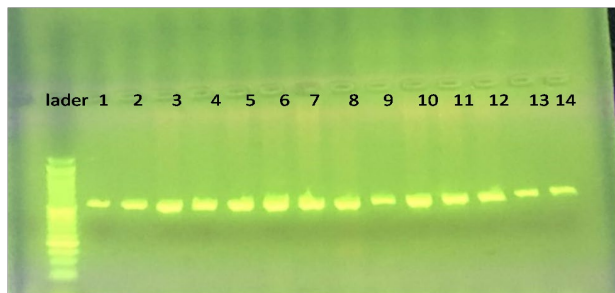


Figure 2. Agarose gel electrophoresis of TGFB1 PCR of experimental group samples.

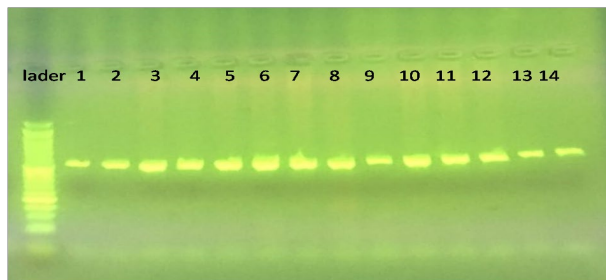


Figure 3. Agarose gel electrophoresis of JAK2 PCR of control group samples.

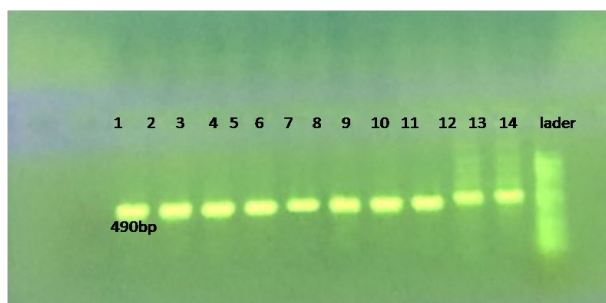


Figure 4. Agarose gel electrophoresis of JAK2 PCR of experimental group samples.

Table 2. Chi-squared test for TGFB1

| | value | df | P-Value |
|--------------------|--------------------|----|---------|
| Pearson Chi-Square | 6.521 ^a | 2 | .038 |
| Likelihood Ratio | 7.768 | 2 | .021 |
| N of Valid Cases | 74 | | |

^a1 cells (16.7%) have expected count less than 5. The minimum expected count is 2.59.

Table 3 Chi-squared test for JAK2 genotype polymorphism

| | value | df | P-Value |
|--------------------|--------------------|----|---------|
| Pearson Chi-Square | 2.292 ^a | 2 | .318 |
| Likelihood Ratio | 2.528 | 2 | .283 |
| N of Valid Cases | 74 | | |

^a1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.86.

According to Table 2, the $P < 0.05$ was acquired for TGFB1, so TGFB1 gene polymorphism is associated with recurrent miscarriage. However, according to Table 3, the $P < 0.05$ was not acquired for JAK2, so the association between JAK2 polymorphism and recurrent miscarriage was not substantial.

Discussion

Polymorphism means natural variants at a specific locus of the genome. Genetic polymorphisms have evolved due to mutations in a locus following evolutionary forces such as natural selection or gene drift, and thus subsequent generations of the population contain these polymorphisms in their genomes (Casillas and Barbadilla, 2017).

Thrombosis, or thrombosis, is called a blood clot inside a blood vessel. This condition is caused by the buildup of blood platelets. This tendency to clot is due to extrinsic factors and genetic factors resulting from changes in clotting mechanisms (Soltan Ghoraie *et al.*, 2007). Thrombophilia and immunologic factors are predisposing factors for recurrent miscarriage.

TGF-B1 is a Th 2 cytokine with multiple biological properties in regulating growth and cell differentiation. Several polymorphisms in the TGF-B1 gene have been identified so far,

some of which have been associated with the severity of the disease (Amani et al. 2003). TGF-B 1 is the most potent immunosuppressive cytokine(Böttinger and Bitzer, 2002). TGFB1 administration can partially protect the organs from the risk of rejection (Blobe *et al.*, 2000).

In this study, TGFB1 gene polymorphism was associated with recurrent miscarriage. But the association between JAK2 polymorphism with recurrent miscarriage was not significant.

TGFB functions as autocrine, paracrine, and sometimes endocrine, and is responsible for regulating various processes including cell proliferation, differentiation, apoptosis, extracellular matrix homeostasis, embryonic development, and wound healing and angiogenesis (Guzeloglu-Kayisli *et al.*, 2009). TGFB1 is a Th2 cytokine with multiple biological properties in regulating the growth of cellular differentiation.

TGF- β is a potent immune-regulating cytokine and regulates the process of implantation and binding of trophoblast cells to the extracellular matrix (Amani *et al.*, 2005).

Amani et al. examined TGFB1 gene polymorphism at (G / A-800) site in recurrent miscarriage (Amani *et al.*, 2005).

A study was conducted in 2005 by Baxter et al. The results showed that JAK2 mutation was found in 71 out of 73 polycythemia patients, 29 out of 51 primary thrombocytopenia patients, and 8 out of 16 idiopathic myelofibrosis patients (Baxter et al., 2005).

In 2012, Rull et. al. wrote an article on abortion genetics and challenges - knowledge and future directions. Related genes for abortion, including TGFB1 and JAK2. Autoimmune factors are produced by the mother's immune response to her antigens. These factors cause the failure of self-tolerance and consequently, the mother's immunologic response to the fetus, which is 30% of cases cause recurrent spontaneous miscarriage (Rull *et al.*, 2012)

Identification of JAK2 gene polymorphisms by sequencing and evaluation of the association between the polymorphism and recurrent

miscarriage were investigated previously (Soltan Ghoraei *et al.*, 2007).

Conclusion

TGFB1 gene polymorphism was associated with recurrent miscarriage. However, the association between JAK2 polymorphism and recurrent miscarriage was not considerable. This finding could help to prevent recurrent miscarriage in Iranian women.

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