J. Appl. Environ. Biol. Sci., 5(12s)912-917, 2015

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

The Association between IL-1 Receptor Antagonist Intron 2 Gene Polymorphisms and Unexplained Recurrent Pregnancy Loss in an Iranian Azari Turkish Population

Seyed Ali RAHMANI[1*], Zeinab PAKNEJAD[1], Masoomeh MOHAMMAD KHANLOU[1], Parvin BASTANI[3], Behzad BARADARAN[2], Zarifeh HASSANZADEH[1]

1Department of Molecular biology, Ahar Branch Islamic Azad University, Ahar, Iran
2 Department of Immunology, Tabriz University of Medical sciences, Tabriz, Iran
3 Medical College, Tabriz University of Medical sciences, Tabriz, Iran

Received: February 21, 2015
Accepted: May 29, 2015

ABSTRACT

Introduction: Recurrent pregnancy loss (RPL) is a heterogeneous condition consisting of three or more consecutive abortions before the 20 weeks of gestation. Cytokines secreted by Th1 cells including IL-1, TNFα and IFNγ, have been described as etiologic factors in RPL. Interleukin 1 receptor antagonist (IL-1RN) is an important anti-inflammatory molecule, which play vital roles in pregnancy. To our knowledge, this was the first report from Iran.
Genotypes and allele distributions for IL-1RN variable number tandem repeat (VNTR) in 100 women with unexplained RPL were compared to those of 100 age and ethnically matched healthy fertile controls with at least two successful pregnancies and no history of pregnancy loss. Genomic DNA was extracted from the whole blood and analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).
Results: No significant association was indicated between these polymorphisms and RPL among Iranian Azeri Turkish women (all P>0.05).
IL-1RN VNTR polymorphisms may not be genetic determinant for RPL.
Conclusion: It is essential to repeat studies and design a more extensive research with a higher number of subjects from different ethnic origins.
KEYWORDS:IL-1 receptor antagonist, polymorphism, unexplained recurrent pregnancy loss

1.INTRODUCTION

Recurrent pregnancy loss (RPL) is a multifactorial event consisting of three or more consecutive abortions [1, 2] which occurs in about 1-2%of women at the reproductive age [3, 4]. The causes are very heterogeneous including genetic, anatomical, chromosomal and endocrinological factors. In addition, environmental factors such as exposure to ethylene oxide and lead have been considered [5]. Sometimes, RPL arises from immunologic problems [6]. Accordingly; there is an argument about the appropriate evaluation and treatment of cases experiencing this event [7].
Anti-inflammatory immune response will increase during normal pregnancy, which seems to bee necessary for fetus protection against maternal pro-inflammatory immune response [8]. Although the adaptive immune system is of great importance in reproductive opportunity via the T cell equilibration in the implantation site, an enhanced activity of innate immune system has been regarded as the main reason of recurrent miscarriage [9, 10].
As a result of the enhanced production of Th1 type cytokines, especially IL-1, TNFα and IFNγ due to the allograft-induced activation and release of substance P during gestation, nitric oxide [NO] substances levels might be elevated, which in turn improve chances of fetus rejection [10, 11].
IL-1 family has an important role in inflammatory reactions. The IL-1 gene cluster locate within 430 kb region on chromosome 2 (2q13-21) [12]. Two types of cytokines constitute the family: pro-inflammatory cytokines [IL-1α, IL-1β] and an anti-inflammatory substance [the IL-1receptor antagonist (IL-1Ra or IL-1RN) [12-16]. The human IL-1RN gene has been determined on the band q14-q21 in which intron 2 encompasses variable number tandem repeat (VNTR) polymorphism with a 86-basepair, and the VNTR sequence is repeated 2 to 6 times. Generally, there are 4, 2, 5, 3 and 6 repeats in allele 1 (IL-1RN*1), allele 2 (IL-1RN*2), allele 3 (IL-1RN*3), allele 4 (IL-1RN*4) and allele 5 (IL-1RN*5), respectively [17, 18]. The production of IL-1RN gene is a 16-18 kDa protein which prohibits the action of IL-1 as a competitive inhibitor and induces no signal transduction [12-16, 19]. As an anti-inflammatory event take places over a normal pregnancy, the levels of IL-1RNwould be raised and an inflammation reaction can be ended [20]. Individual susceptibility to disease would be determined the levels of cytokines production which is affected by cytokine gene polymorphisms [21]. It has been suggested that IL-1 plays a crucial role in embryonic development through the regulation of blastocyst implantation and the inducement of the endometrial leukemia inhibitory factor (LIF) production. Moreover, gene expression and synthesis of IL-1RN have been established in the

Corresponding author: Seyed Ali Rahmani, Department of Molecular biology, Ahar Branch Islamic Azad University, Ahar, Iran;
Email: a-rahmani@iau-ahar.ac.ir

dividing embryo [22, 23] which is considered to be associated with RPL [18].
In this regard, the object of the study was to investigate the association between IL-1RN VNTR polymorphism and RPL in Iranian Azeri Turkish women experiencing RPL.

MATERIAL & METHOD

A case controlled, study was conducted to determine the association of RPL with IL-1RN VNTR polymorphism. Cases were 100 women who had suffered at least three pregnancy losses [mean 5, range 3-7] and showed normal karyotypes. No chromosomal aberration and uterine anatomical abnormalities as well as infections related miscarriages were found. As a whole, there were no identifiable causes for RPL and the miscarriages were thus categorized as unexplained. The control group consisted of 100 healthy age and ethnically matched women in childbearing ages who delivered at least two healthy, term infant with no history of pregnancy loss. All women were of Iranian Azeri Turkish origin, with the mean age of 32[range 21-45] and 35.5 (range 25-47) for case and control groups, respectively.
All individuals were informed about the study and the samples were collected with their agreement.5ml of blood samples were collected into tubes containing EDTA as an anticoagulant. Genomic DNA was extracted from whole blood by using proteinase K method[…]. Nanodrop instrument was used to determine the quality and quantity of each DNA sample. The results were confirmed by electrophoresis on the 1% agarose gel (Figure 1). To amplify the second intron of the 86-bp VNTR containing gene of IL-1RN, DNA was amplified by polymerase chain reaction (PCR): Initial denaturation for 1 minute at 94°C, followed by 35 cycles of denaturation (1 minute at 94°C), annealing (45 seconds at 55°C), extension (45 seconds at 72°C), and a final extension for 5 minutes at 72°Cusing the following primers:
Forward: 5' CTCAGCAACACTCCTAT 3'
Reverse: 5' TCCTGGTCTGCAGGTAA 3'
Electrophoresis was performed on 1.5% gelatine by ethidium bromide to determine the size of amplified PCR products (Figure The gels were photographed by using the gel documentation instrument. In addition, a 50 bp size marker was loaded onto the gel. The size of amplified alleles were 410 bp, 240bp, 500bp, 325bp and 595 bp, respectively.
The difference of IL-1RN genotype and frequencies of allele between case group and control group was analyse with chi-square test. association between allele frequencies and RPL was measured with The odds ratio (OR). All P values were two-tailed and 95% confidence intervals (CI) were calculated. P values <0.05 were considered statistically significant.

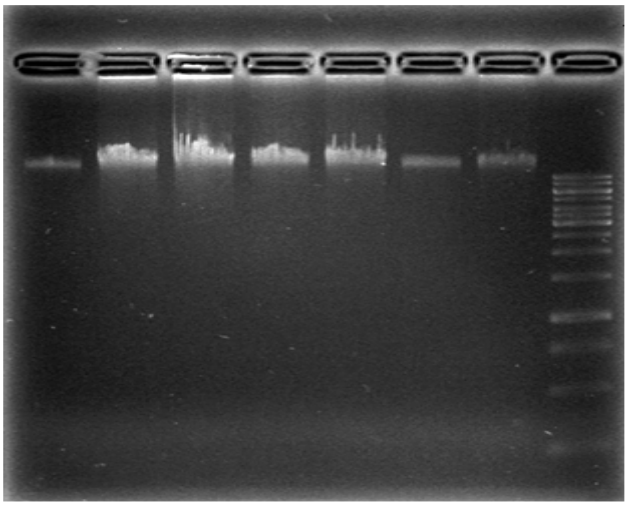

Fig 1. DNA Electrophoresis on 1% agarose gel. 8 shows 250 bp DNA ladder

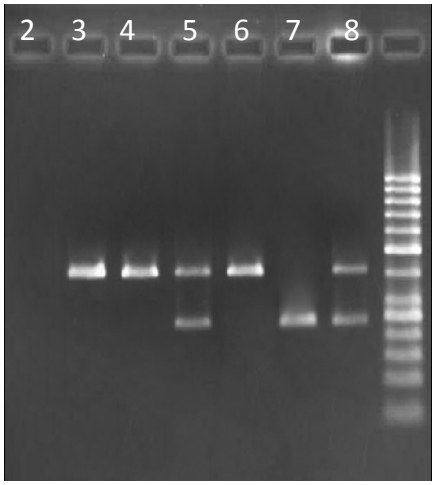

Figure 2. 1. negative control/ 2,3, 5. Allele 1/ 6. allele 2/ 4, 7. Alleles 1 and 2/ 8. DNA ladder [50bp]

RESULTS

The IL-1RN polymorphisms in 100 females with unexplained RPL were compared to 100 matched healthy females in terms of age and ethnicity who delivered at least two healthy, term infants with no history of pregnancy loss. No statistically significant difference were observed between the study and control groups with respect to all genotypes. For instance, according to allele 1 homozygotes (IL-1 RN1/1; 53% vs. 51%; P: 0.88; OR: 1/083; 95% CI: 0/599-1/961) and allele 1 heterozygotes (IL-1 RN1/2: 35% vs. 28%; P: 0.36; OR: 1.385; 95% CI: 0/728-2/636), the dispensation of genotype prevalences was not significantly different in the RPL patients from the healthy subjects (Table1).

The allelic frequency of women experiencing RPL and their healthy controls and the associated ORs are shown in table 2. As can be found from the table, there is no important differences among the prevalence of IL-1RN alleles in the control group compared to case.

The most frequent allele in patients and controls were IL1RN*1 which was higher among the patients than controls. However, no significant difference was observed (73.5% vs. 69%; P: 0.37; OR: 1/969-0/789). On the other side, noIL1RN*5 was found and IL1RN*4 allelic frequency was only 0.5% in both groups.

Table 1. frequency of the IL-1RN polymorphism Genotype among Iranian Azari Turkish RPL patients and healthy fertile women.

GenotypeIL-1RN	Patients n=100||	Controls n=100||	P value	Odds ratio 95%CI||
IL-1RN 1/1	53 [53%]	51 [51%]	0/88	**1/083 [0/599-1/961]**
IL-1RN 1/2	35 [35%]	28 [28%]	0/36	**1/385 [0/728-2/636]**
IL-1RN 1/3	5 [5%]	7 [7%]	0/76	**0/699 [0/185-2/568]**
IL-1RN1/4	1 [1%]	1 [1%]	1/00	**1/000 [0/027-37/156]**
IL-1RN 2/2	4 [4%]	10[10%]	0/16	**0/375 [0/095-1/363]**
IL-1RN 2/3	2 [2%]	2 [2%]	1/00	**1/000 [0/098-10/167]**
IL-1RN 3/3	0	1 [1%]	1/00	**0/000 [0/000-17/447]**
IL-1RN 4/4	0	0	1/00	-
IL-1RN 5/5	0	0	1/00	-

Table 2. frequency of Allel of the IL-1RN polymorphism among Iranian Azari Turkish RPL patients and healthy fertile women

AlleleIL-1RN	Patients [%] n=100||	Controls [%] n=100||	P value	Odds ratio 95%CI||
IL-1RN 1	73/5	69	0/37	**1/246 [1/969-0/789]**
IL-1RN 2	22/5	25	0/63	**0/871 [1/4217-0/535]**
IL-1RN 3	3/5	5/5	0/47	**0/623 [1/780-0/213]**
IL-1RN 4	0/5	0/5	1/00	**1/00 [36/817-0/027]**
IL-1RN 5	0	0	1.00	-

DISCUSSION

To date, varieties of genetic disorders have been studied to determine the genetic cornerstone of RPL [2, 25-28]. Moreover, several studies have been carried out to identify an association between IL-1RN polymorphisms and RPL in different conditions. However, due to conflicting reports, we decided to investigate this polymorphism in our ethnic population. It is worth mentioning that this was the first research on RPL and IL-1RN polymorphisms in Iran.

Karthukorpi et al, reported different frequency of IL-1RN and -1RN in women suffering from recurrent spontaneous abortion compared with healthy subjects were not considerably different, while the IL-1RN*3 was significantly higher in patients than controls[8].

According to IL-1RN and IL-1RN, the results of our investigation were in concordance with Karthukorpi, but it was not confirmed their findings about IL-1RN*3. Of note, despite the higher frequency of allele 2 homozygotes [IL-1 RA2/2] in healthy subjects than patients [10% vs. 4%], its association with RPL was not important [P: 0.16]. Similar to this result were obtained by Die et al, who stated IL-1RN*2is not related to idiopathic recurrent spontaneous abortion in the Chinese Han population[29].Vargas-Alarcon et al. Compared the prevalences of IL-6, IL-1RN, INF-γ, IL-10, and TNF-α gene polymorphisms and found a significantly different distribution of these in the Mexican population [30]. Linjawi et al. [21], in astudy compared IL- 1RN genotype of 206 women that they had several miscarriage with control group. Results showed no noticeable differences between their frequencies. Similar to Linjawi [21], Agrawal [31] and Traina [32] studies as well as Levrant et al. [18], we found no remarkable differences between the frequencies of IL-1RN polymorphisms in females experiencing RPL and their controls who were Iranian Azeri Turkish women with at least two healthy, term infants. Likewise, such a result was obtained by Jaaskelainen et al. [33]. In contrast, Perni et al. [34] Explained an increase in spontaneous abortions in the presence offetalIL-1RN*1 [34].

This study provides new insight toward the established multigenetic context of RPL in which IL-1RN polymorphisms did not play a role in the occurrence of RPL in Iranian population from Azeri Turkish origin, so normal pregnancy will not be affected by their presence. As has been mentioned in the literature, the controversial reports from different studies can be satisfied by various reasons [18]. Finding of this study will help us to determin women who are at the risk of pregnancy loss.Further more [16, 18, 39].

Conclusion: the exactrole of IL-1RN polymorphisms in RPL is not still fully understood. So, it is essential to repeat studies with a higher number of subjects from different ethnic origins to find the effect of IL-1RN polymorphisms in pregnancy loss.

Acknowledgements

This work was supported by research grants from the Ahar Branch, Islamic Azad University for research with title: The effects of tumor necrosis factor-α, IL-1 receptor antagonist and Interleukin-1β genes polymorphisms on recurrent abortion in Azari women.

REFERENCES

1. López Ramírez Y, Vivenes M, Miller A, Pulido A, López Mora J, Arocha-Piñango CL, et al. Prevalence of the coagulation factor XIII polymorphism Val34Leu in women with recurrent miscarriage. Clinica chimica acta.;374[1]:69-74. **[2006]**
2. Glueck CJ, Wang P, Bornovali S, Goldenberg N, Sieve L. Polycystic ovary syndrome, the G1691A factor V Leiden mutation, and plasminogen activator inhibitor activity: associations with recurrent pregnancy loss. Metabolism, clinical and experimental.;52[12]:1627-32. **[2003]**
3. Omwandho CA, Tinneberg HR, Tumbo-Oeri AG, Roberts TK, Falconer J. Recurrent pregnancy losses and the role of immunotherapy. Archives of Gynecology and Obstetrics.;264[1]:3-12. [**2000]**
4. Brenner B. Inherited thrombophilia and pregnancy loss. Best Practice & Research Clinical Haematology.;16[2]:311-20. **[2003]**
5. Calderwood CJ. Thromboembolism and thrombophilia in pregnancy. Current Obstetrics & Gynaecology.;16[6]:321-6. **[2006]**
6. Scott J. Immunotherapy for recurrent miscarriage. The Cochrane Library. **[2006].**
7. Coulam CB, Clark DA, Beer AE, Kutteh WH, Silver R, Kwak J, et al. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. American Journal of Reproductive Immunology.;38[2]:57-74. **[2011]**
8. Karhukorpi J, Laitinen T, Kivelä H, Tiilikainen A, Hurme M. IL-1 receptor antagonist gene polymorphism in recurrent spontaneous abortion. Journal of Reproductive Immunology.;58[1]:61-7. **[2003]**
9. Robertson SA. Immune regulation of conception and embryo implantation—all about quality control? Journal of Reproductive Immunology.;85[1]:51-7. **[2010]**

10. Wilczyński JR. Immunological Analogy Between Allograft Rejection, Recurrent Abortion and Pre-Eclampsia–the Same Basic Mechanism? Human immunology.;67[7]:492-511. **[2006]**
11. Thellin O, Heinen E. Pregnancy and the immune system: between tolerance and rejection. Toxicology.;185[3]:179-84. **[2003]**
12. Zhou L, Cai J, Liu G, Wei Y, Tang H. Associations between Interleukin-1 Gene Polymorphisms and Coronary Heart Disease Risk: A Meta-Analysis. PLOS ONE. 2012;7[9]:e45641.
13. Banerjee M, Saxena M. Interleukin-1 [IL-1] family of cytokines: Role in Type 2 Diabetes. Clinica chimica acta.;413[15–16]:1163-70. **[2012]**
14. Ross R, Grimmel J, Goedicke S, Möbus AM, Bulau AM, Bufler P, et al. Analysis of nuclear localization of interleukin-1 family cytokines by flow cytometry. Journal of Immunological Methods. **[2012].**
15. Arend WP. Interleukin-1 receptor antagonist. Advances in immunology.;54:167-227. **[1993]**
16. Volarevic V, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. Interleukin-1 receptor antagonist [IL-1Ra] and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. Autoimmunity.;43[4]:255-63. **[2009]**
17. Murtha AP, Nieves A, Hauser ER, Swamy GK, Yonish BA, Sinclair TR, et al. Association of maternal IL-1 receptor antagonist intron 2 gene polymorphism and preterm birth. American Journal of Obstetrics and Gynecology.;195[5]:1249-53. **[2006]**
18. Levrant S, Coulam CB, Jeyendran RS. Interleukin 1 receptor antagonist gene polymorphisms are not risk factors for recurrent pregnancy loss: evaluation of couples. American Journal of Reproductive Immunology.;60[3]:224-8. **[2008]**
19. Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Human genetics.;91[4]:403-4. **[1993]**
20. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clinical infectious diseases.;34[2]:204-9. **[2002]**
21. Linjawi S, Li TC, Laird S, Blakemore A. Interleukin-1 receptor antagonist and interleukin-1 beta polymorphisms in women with recurrent miscarriage. Fertility and Sterility.;83[5]:1549-52. **[2005]**
22. Nilkaeo A, Bhuvanath S. Interleukin-1 modulation of human placental trophoblast proliferation. Mediators of inflammation. 2006;**[2006].**
23. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annual review of immunology.;27:519-50. **[2009]**
24. Culić V, Konjevoda P, Mise K, Kardum G, Matijević T, Pavelić J. Genitourinary diseases prior spontaneous abortion as a risk factor for recurrent pregnancy loss. Collegium Antropologicum.;33[1]:187-92. **[2009]**
25. Dossenbach-Glaninger A, van Trotsenburg M, Dossenbach M, Oberkanins C, Moritz A, Krugluger W, et al. Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clinical chemistry.;49[7]:1081-6. **[2003]**
26. Souza S, Ferriani R, Pontes A, Zago M, Franco R. Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion. Human Reproduction.;14[10]:2448-50. **[1999]**
27. Sierra S, Stephenson M, editors. Genetics of recurrent pregnancy loss. Seminars in reproductive medicine; **[2006].**
28. Kaur A. Recurrent pregnancy loss: TNF-α and IL-10 polymorphisms. Journal of human reproductive sciences.;4[2]:91. **[2011]**
29. Dai M, Pan Y, Xu D, Chen X, Qian R, Chen D, et al. IL-1 receptor antagonist gene polymorphism in idiopathic recurrent spontaneous abortion in a Chinese Han population. International journal of immunogenetics.;37[5]:393-6. **[2010]**
30. Vargas-Alarcon G, Ramírez-Bello J, Juárez-Cedillo T, Ramírez-Fuentes S, Carrillo-Sánchez S, Fragoso JM. Distribution of the IL-1RN, IL-6, IL-10, INF-γ, and TNF-α Gene Polymorphisms in the Mexican Population. Genetic Testing and Molecular Biomarkers. **[2012].**
31. Agrawal S, Parveen F, Faridi RM, Prakash S. Interleukin-1 gene cluster variants and recurrent pregnancy loss among North Indian women: retrospective study and meta-analysis. Reproductive BioMedicine Online.;24[3]:342-51. **[2012]**
32. Traina É, Daher S, Moron AF, Sun SY, Franchim CS, Mattar R. Polymorphisms in VEGF, progesterone receptor and IL-1 receptor genes in women with recurrent spontaneous abortion. Journal of Reproductive Immunology.;88[1]:53-7. **[2011]**

33. Jaaskelainen E, Keski-Nisula L, Toivonen S, Paattiniemi EL, Helisalmi S, Punnonen K, et al. Polymorphism of the interleukin 1 receptor antagonist [IL1Ra] gene and placental abruption. Journal of Reproductive Immunology.;79[1]:58-62. **[2008]**
34. Perni SC, Vardhana S, Tuttle SL, Kalish RB, Chasen ST, Witkin SS. Fetal interleukin-1 receptor antagonist gene polymorphism, intra-amniotic interleukin-1β levels, and history of spontaneous abortion. American Journal of Obstetrics and Gynecology.;191[4]:1318-23. **[2004]**
35. Unfried G, Tempfer C, Schneeberger C, Widmar B, Nagele F, Huber JC. Interleukin 1 receptor antagonist polymorphism in women with idiopathic recurrent miscarriage. Fertility and Sterility.;75[4]:683-7. **[2001]**
36. Tountas NA, Casini–Raggi V, Yang H, Di Giovine FS, Vecchi M, Kam L, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. Gastroenterology.;117[4]:806-13. **[1999]**
37. Francis SE, Camp NJ, Dewberry RM, Gunn J, Syrris P, Carter ND, et al. Interleukin-1 receptor antagonist gene polymorphism and coronary artery disease. Circulation.;99[7]:861-6. **[1999]**
38. Barton PT, Gerber S, Skupski DW, Witkin SS. Interleukin-1 receptor antagonist gene polymorphism, vaginal interleukin-1 receptor antagonist concentrations, and vaginal Ureaplasma urealyticum colonization in pregnant women. Infection and immunity.;71[1]:271-4. **[2003]**
39. Stricker RB, Steinleitner A, Bookoff CN, Weckstein LN, Winger EE. Successful treatment of immunologic abortion with low-dose intravenous immunoglobulin. Fertility and Sterility.;73[3]:536. **[2000]**