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Effects of IL-1 receptor antagonist *intron 2* gene polymorphisms on recurrent pregnancy loss in Iranian population.

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Abstract

Introduction: Recurrent Pregnancy Loss (RPL) is a heterogeneous disease which consisting of three or more successive abortions before 20 weeks of pregnancy. The cytokines that secreted by Th1 cells (IL-1, TNFα and IFNγ) were described as etiologic factors in RPL. The aim of this study was investigate to association between recurrent pregnancy loss and IL-1 receptor antagonist gene (*IL-1RN*) *intron 2* polymorphism (86-bp VNTR) in Iranian Azeri and Persian women.

Materials and methods: Genotype and allele distribution were studied in 280 Persian women (140 case and 140 control) and 200 Azeri women (100 case and 100 control). Case group were included women with least three RPL and control group were included healthy women with at least two successful deliveries. Genomic DNA was extracted from the whole blood and polymorphism analysis was performed by Polymerase Chain Reaction (PCR) method.

Results: No significant association was observed between *IL-1RN* 86-bp VNTR polymorphism in *intron 2* and RPL among Iranian Persian and Azeri women.

Conclusion: *IL-1RN* VNTR polymorphism may not be a genetic factor for RPL. However investigation of *IL-1RN* polymorphism was recommended in other populations and patients with recurrent pregnancy loss.

Keywords: *IL-RN*, Polymorphism, Recurrent pregnancy loss.

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Introduction

Recurrent Pregnancy Loss (RPL) is a multi-factorial syndrome consists of three or more successive abortions [1-3] and is a serious reproductive problem in 1-5% of reproductive age women [4,5]. The causes for this syndrome are very different, such as genetical, anatomical, chromosomal and endocrinological agents. Also environmental agents are important, which including disposal to ethylene oxide and lead [6]. In several conditions, RPL appear from immunological problems [7]. Since there is a reasoning about the suitable evaluation and treatment of cases experiencing this disease [8].

Anti-inflammatory immune response during pregnancy is normal and necessary for embryo conservation against maternal pro-inflammatory immune response [9]. The increased production of Th1 type cytokines, particularly IL-1, TNFα and IFNγ, due to allograft induced activation and release of material P during pregnancy, nitric oxide and other toxic material might be elevated, that in turn improve pregnancy loss chances [10,11].

The *IL-1* gene family has an important effect on inflammatory response. *IL-1* cluster has located within 430 kilo base area on the chromosome 2 (2q13-21) [12]. There are two types of cytokines family, including pro-inflammatory cytokines (IL-1α, IL-1β) and an anti-inflammatory material (IL-1Ra or *IL-1RN*) [12,13]. Human *IL-1RN* gene has been defined in the q14-q21 condition, which *intron 2* encompasses VNTR polymorphism with an 86-base pair and the VNTR sequence was repeated 2 to 6 times. Usually, there are 4, 2, 5, 3 and 6 repetition 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 [14,15]. The product of *IL-1RN* gene is a protein with 16-18 kDa weight that inhibits the function of IL-1 as a competitive inhibitor and induces no signal transduction [12,13,16]. As an anti-inflammatory event take places over an ordinary gestation, the levels of *IL-1RN* would be raised and an inflammation response can be terminated [17]. Person's susceptibility to this syndrome would be defined by the amount of cytokines product that was affected through cytokine gene polymorphisms [18]. Also suggested that *IL-1* gene play an important role in fetal development by regulation of blastocyst implantation and indication the production of the endometrial leukemia inhibitory factor [15]. In addition, expression and synthesis of *IL-1RN* gene have been established in the dividing fetus [19,20], that was demonstrated to be relation with RPL [15]. Therefore the aim of this study was investigate to association between recurrent pregnancy loss and IL-1 receptor antagonist gene *intron 2* polymorphism (86-bp VNTR) in Iranian Azeri and Persian women.

Materials and Methods

This case-control study was performed to define association between RPL and *IL-1RN* VNTR polymorphism in Iranian Persian and Azeri women. The cases group were included 140 (Persian) and 100 (Azeri) women who had suffered at least three pregnancy losses (mean 5, range 3-7) and showed normal karyotypes. No were found chromosomal aberration and uterine anatomical abnormalities as well as infections related miscarriages. The control group were included 140 (Persian) and 100 (Azeri) healthy age and ethnically matched adult women with at least two successful delivery and with no history of pregnancy loss. All women were selected from Iranian Tehran and Azerbayjan origin, with the mean age of 32 (range 21-45) and 35.5 (range 25-47) for case and control groups, respectively (Table 1).

The women were knowledgeable about this study and the blood samples were prepared with their agreement. Initially 5 ml of blood samples were taken and was transferred into tubes contains EDTA. DNA extraction was performed by proteinase K method. In order to determination the quality and quantity of DNA samples was used from Nanodrop instrument. Polymerase Chain Reaction (PCR) used to amplify *IL-1RN* gene 86-bp VNTR polymorphism in *intron 2*: initial denaturation (1 minute at 94°C), denaturation (1 minute at 94°C, 35 cycles), annealing (45 sec at 55°C), extension (45 sec at 72°C) and final extension (5 min at 72°C) by using this primers: 5'-CTCAGCAACACTCCTAT-3' (forward) and 5'-TCCTGGTCTGCAGGTAA-3' (reverse).

To determination the size of PCR production was performed electrophoresis on 1.5% agarose gel that was stained by ethidium bromide. Gel documentation instrument was used for photograph from the agarose gel. Furthermore a marker whit 50 bp was loaded in the gel.

The chi-square test was performed to analyse of the *IL-1RN* genotype and allele frequencies (SPSS software version 17). The Odds Ratio (OR) was used to measure of association between allele frequencies and RPL. P-values were two-tailed and were calculated 95% confidence intervals. P-values with <0.05 were considered statistically significant.

Results

The *IL-1RN* polymorphisms were studied in women with unexplained RPL and healthy women from Azeri and Persian region. The results were confirmed by electrophoresis on the 1.5% agarose gel. The sizes of amplified alleles were 410 bp, 240 bp, 500 bp, 325 bp and 595 bp.

Table 1. *Demographic variable of Azeri and Persian women in patient and control groups.*

Variable	Tehran (n=140)		P-value	Azerbaijan (n=100)		P-value
	Case	Control		Case	Control	
Age 20-25 years	49 (35%)	39 (27.8%)	0.101	29 (29%)	34 (34%)	0.32
26-30 years	70 (50%)	63 (45%)		51 (51%)	47 (47%)	
31-35 years	21 (15%)	38 (27.2%)		20 (20%)	19 (19%)	
BMI (Kg/m^2)	25.16 ± 3.16	23.44 ± 3.10	0.02	26.03 ± 3.10	23.90 ± 3.11	0.101
Education						
Diploma and less	61 (43.5%)	53 (37.8%)	0.021	53 (70%)	63 (63%)	0.021
High educated	39 (56.5%)	47 (62.2%)		47 (30%)	37 (37%)	

There was no statistically significant difference between case and control groups in Azeri women. The genotype and allele frequencies of control and case groups and also associated ORs were shown in Tables 2 and 3. According to Table 2, there was no significant difference between the frequency of *IL-1RN* alleles in control and case groups. The most allele frequency was the *IL1RN*1* in case and control women, but was more in the case group. However, was not observed significant difference (73.5% *vs.* 69%; P: 0.37; OR: 1/969-0/789). In other hand, was not found *IL1RN*5* allele in Azeri women, but *IL1RN*4* allelic frequency was 0.5% in both Azeri control and case groups.

Persian women results in all cases as well as Azeri women and was no significant association between this polymorphism and unexplained recurrent pregnancy loss. The most allele frequency was the *IL1RN*1* in case and control groups, but was more in the case group (Table 2). However, was not observed significant difference (77.0% *vs.* 71%; P: 0.43; OR: 1/999-0/841). Also allele 1 homozygotes was IL-1 RN1/1; 54% *vs.* 49%; P: 0.78; OR: 1/04; 95% CI: 0/7-1/0 and allele 1 heterozygotes was IL-1 RN1/2: 37% *vs.* 38%; P: 0.40; OR: 1.5; 95% CI: 0/821-2/739 in Tehran women, but allele 1 homozygotes was IL-1 RN1/1; 53% *vs.* 51%; P: 0.88; OR: 1/083; 95% CI: 0/599-1/961 and allele 1 heterozygotes was L-1 RN1/2: 35% *vs.* 28%; P: 0.36; OR: 1.385; 95% CI: 0/728-0.0212/636 in Azeri women.

Table 2. *Genotype frequencies of the IL-1RN polymorphism among Iranian case and control women.*

Tehran (n=140)				Azerbaijan (n=100)		
L-1RN genotype	**Case**	**Control**	**P-value**	**Case**	**Control**	**P-value**
IL-1RN 1/1	54%	49%	0/91	53%	51%	0/88
IL-1RN 1/2	37%	38%	0/95	3%	28%	0/36
IL-1RN 1/3	3%	5%	0/87	5%	7%	0/76
IL-1RN1/4	1%	2%	91	1%	1%	1/00
IL-1RN 2/2	3%	4%	0/88	4%	10%	0/16
IL-1RN 2/3	1%	2%	0/93	2%	2%	1/00
IL-1RN 3/3	1%	0%	1/00	0%	1%	1/00
IL-1RN 4/4	0%	0%	1/00	0%	0%	1/00
IL-1RN 5/5	0%	0%	1/00	0%	0%	1/00

Table 3. *Allelic frequencies of the IL-1RN polymorphisms among Iranian case and control women.*

Tehran (n=140)				Azerbaijan (n=100)		
IL-1RN allele	**Case**	**Control**	**P-value**	**Case**	**Control**	**P-value**
IL-1RN 1	77%	71%	0/38	73/5%	69%	0/37
IL-1RN 2	18%	23%	0/59	22/5%	25%	0/63
IL-1RN 3	4%	4%	1/00	3/5%	5/5%	0/47
IL-1RN 4	1%	1/5%	0/29	0/5%	0/5%	1/00
IL-1RN 5	0%	0/5%	0/31	0%	0%	1/00

Discussion

So far several polymorphisms including of PAI-1 4G/5G and FXIII Val34Leu, the G1691A and factor V Leiden mutation have been investigated to determine the genetic basis of RPL [2,21,22]. In addition, many studies have been performed to determine an association between *IL-1RN* polymorphisms and RPL. However, due to inconsistent reports, we investigate *IL-1RN* polymorphism relation in two ethnic populations in Iran.

The *IL-1RN* mediated inflammatory processes have been proposed to be involved in the pathogenesis of pregnancy complications [15]. The *IL-1RN* was expressed by blastocysts and plays an important role in trophoblast growth and invasion [23]. The IL-1RN cytokine is a negative regulator for inflammatory cytokines of IL-1a and IL-1b [24]. Patients with the history of recurrent pregnancy loss have high proinflammatory response compared to normal pregnant women which has normal anti-inflammatory response [25]. Therefore decrease of *IL-1RN* led to increase the inflammatory processes and to be involved in pregnancy loss.

Karthukorpi et al. showed that the frequency differences of *IL-1RN*1* and *IL-1RN*2* were not very different in women with RPL in compared with healthy women, while the frequency of *IL-1RN*3* was significantly higher in patient women than healthy women [9]. According to frequency of *IL-1RN*1* and *IL-1RN*2*, the results of present study were in agreement with Karthukorpi study, but it was not agreement about *IL-1RN*3*. Despite the higher frequency of *IL-1RN*2* allele homozygotes (*IL-1RN*2/2) in control group than case group (10% *vs.* 4%), was not significantly associated with RPL. Dai et al. study results were obtained that *IL-1RN*2* were not associated with idiopathic RPL in the Chinese Han population [26]. Linjawi et al. compared 206 women with recurrent miscarriage with their controls in terms of *IL- 1RN*2* alleles and found no significantly differences between their frequencies [18]. Similar to Linjawi [18], Agrawal [27], Traina [28] and Levrant [15] studies we found no significantly differences between the frequency of *IL-1RN* polymorphism in Iranian Tehran and Azerbayjan women with RPL and their controls.

This study showed that *IL-1RN* polymorphisms did not association with RPL in Iranian population from Tehran and Azerbayjan origin. The controversial reports from different studies can be satisfied by various reasons, such as the differences in the selected study groups [15], different sample sizes [28], accidental events, other involved genes and the mechanisms regulating the production of such cytokines [29], the influence of ethnic heterogeneity [29-31] and the different environmental factors [32].

It is believed that finding the association of gene polymorphisms and unexplained abortions will provide us a better understanding about patient's problem or determination of women who are at the risk of pregnancy loss. Furthermore, identification of gene polymorphisms would change the treatment strategy of the subjects [13,15,33].

In conclusion, the exact role of *IL-1RN* polymorphisms in RPL is not still fully understood. So, to reach the more accurate results and to define the specific function of *IL-1RN* polymorphisms in pregnancy loss, it is essential to repeat studies and design a more extensive research with a higher number of subjects from different ethnic origins.

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Declaration of Interest

The authors declare that they have no conflicts of interest.

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