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The effects of tumor necrosis factor-α (TNF-α) and IL-1 receptor antagonist (IL-1Ra) polymorphisms on recurrent abortion in Azari women

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ABSTRACT

Objectives: Recurrent pregnancy loss (RPL) is a heterogeneous condition consisting of three or more consecutive abortions before the 20 weeks of gestation. The tumor necrosis factor alpha (TNF-α) gene plays a crucial role in immunology and inflammation responses. Interleukin 1 receptor antagonist (IL-1RN) is an important anti-inflammatory molecule which plays important roles in pregnancy. The aim of this study was to investigate effects of TNF-α and IL-1Ra polymorphisms on RPL in Azari women.

Material and methods: The study participants consisted of 100 women with RPL from Iranian Azeri Turkish origin. The control group comprised 100 age and ethnically matched healthy women in the reproductive age. Genomic DNA was extracted from the whole blood and genotype determinations were performed using PCR amplification followed by restriction fragment length polymorphism (RFLP) analysis.

Results: No significant association was indicated between IL-1Ra and RPL among Iranian Azeri Turkish women. Unlike the homozygous state, significantly higher frequency of -857 C/T variant was seen in RPL patients than control subjects. Significantly lower frequency of wild type genotype was observed in RPL patients than of controls. Any association was found between the other TNF-α polymorphisms and RPL.

Conclusions: TNF-α -857 C/C variant might represent protective effect against RPL and the -857 C/T variant might be a genetic risk factor for the occurrence of RPL. Invariant differences in the prevalence of -511 C/T and -31 C/T polymorphisms and IL-1RN VNTR between RPL patients.

Key words: TNF-α, IL-1RN, polymorphism, recurrent pregnancy loss

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INTRODUCTION

Recurrent pregnancy loss (RPL) is a multifactorial event consisting of three or more consecutive abortions before 20 weeks of gestation [1] which occurs in approximately 1–2% of women at the reproductive age [2]. 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 [3]. In some cases, RPL arises from immunologic problems [4] and coagulation factors mutations [5]. Furthermore, hyperprolactinemia and hyperhomocysteinuria [6] as well as infectious agents [7] have been identified. However, it has been proven that the reason is unknown for approximately 40–50% of patients [8]. According to these heterogeneous causes, the appropriate evaluation of patients is necessary to explore the exact underlying pathophysiologic mechanisms; thereby the cases experiencing this event would receive a better treatment [6].

It is obvious that T helper 1 (Th1) and T helper 2 (Th2) are the major components of immune system and their functional balance has great importance for a normal pregnancy [9]. Th2 type cytokines including Il-4, IL-5, IL-6 and IL-10 are involved in normal pregnancy. In contrast, Th1 produces proinflammatory cytokines containing IL-1, IFN-γ and TNF-α which are supposed to be related to poor pregnancy outcome [10]. Tumor necrosis factor alpha (TNF-α)

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is an important proinflammatory cytokine secreted by monocytes/macrophages, B cells, natural killer (NK) cells and antigen-stimulated T cells which is located in 6p21.3, the region of human leukocyte antigen (HLA) class III [1]. It has been described that this cytokine is effective on the process of RPL [11]. TNF-α production, secretion and activity are influenced by eight polymorphisms which occur in the promoter region as follows: 1031T/C, 863C/A, 857C/T, 575G/A, 376G/A, 308G/A, 244G/A, and 238G/A (1). There are conflicting reports about the association of these polymorphisms and RPL in different population. In this regard, some literature showed a positive association [12], but a negative relationship was reported by others [13]. The presence of six TNF-α polymorphisms at the positions -1031T/C, -863C/A, -857C/T, -376G/A, -308G/A and -238G/A were compared in the present study to make a better decision about the association of TNF-α polymorphisms in RPL.

IL-1 family has an important role in inflammatory reactions. The IL-1 gene cluster has located within 430 kb region on chromosome 2 (2q13-21) [14]. Two types of cytokines constitute the family: pro-inflammatory cytokines (IL-1α, IL-1β) and an anti-inflammatory substance the IL-1 receptor antagonist (IL-1Ra or IL-1RN) [14]. 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 an 86-base pair, 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 [15]. 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 [14]. As an anti-inflammatory event takes place over a normal pregnancy, the levels of IL-1RN would be raised and an inflammation reaction can be terminated [16]. Individual susceptibility to disease would be determined by the levels of cytokines production which is affected by cytokine gene polymorphisms [17]. 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 dividing embryo [18], which is considered to be associated with RPL [15].

To our knowledge, there have been no reports on the frequency of these in the TNF-α and IL-1Ra gene and their relationship with RPL in Iranian population. Furthermore, conflicting findings have been mentioned in different studies. Hence, to shed new insight on the mechanisms of the RPL, allele frequencies and genotype distributions of TNF-α and IL-1Ra gene polymorphisms and their association with RPL were investigated in cases and the results were compared with those of the age and ethnically matched healthy fertile subjects from Iranian Azeri Turkish population.

MATERIAL AND METHODS

A case-control study was conducted to determine the association of RPL with IL-1RN VNTR and TNF-α (-1031T/C, -863C/A, -857C/T, -376G/A, -308G/A and -238G/A) polymorphism. A total of 100 patients and 100 healthy controls who attended the educational hospitals were related to Tabriz University of medical sciences between 2011 and 2012. Were included in this retrospective case control study. Subjects were women aged 21–45 years who had experienced at least three continuous abortions before 20 weeks of conception. The patients' karyotypes and the structure of uterine were normal, no infection related miscarriages were detected and any other identifiable causes were figured out. So, the events were classified as unexplained pregnancy loss. The age matched control subjects were selected from healthy fertile women with at least two live births and no history of pregnancy loss. In order to prevent the epidemiological bias, all recruited subjects had the same ethnicity and belonged to Iranian Azeri Turkish origin. All participants were informed about the study and signed a consent form.

In DNA extraction stage blood samples (5 mL) from antecubital vein were collected into tubes containing EDTA as an anticoagulant. Genomic DNA was extracted using the proteinase K method. Nanodrop instrument was employed to determine the quality and quantity of each DNA sample and electrophoresis on the 1% agarose gel was performed to confirm the results. The extracted DNA samples were stored at -20°C until analyzed. In the following, experiments were conducted respectively:

— TNF-α: DNA samples were amplified and investigated for six polymorphisms in the promoter region of TNF-α gene (-1031T/C, -863C/A, -857C/T, -376G/A, -308G/A and -238G/A) using polymerase chain reaction (PCR): 1 cycle of initial denaturation in 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 1 minutes), annealing (for 45″), extension (72°C for 45″) and a final extension at 72°C for 5 minutes. This protocol was used for all polymorphisms but the annealing temperatures for each pair of primers were different (Table 1). The PCR products were electrophoresed on 1.5% agarose gel stained by ethidium bromide. Following amplification reaction, the PCR products were digested through restriction fragment length polymorphism (RFLP) analysis using the appropriate restriction endonucleases. Then, electrophoresis of the digested products was performed on 3% agarose gel stained by ethidium bromide. The size of bands was estimated by using a 50 base pair molecular weight marker. A gel documentation instrument was used for visualizing the bands of PCR and digested products of RFLP analysis.

Table 1. Primer sequences used for detection of TNF-α gene polymorphisms

Polymorphism	Primer sequences	Annealing temperature	Size of amplified product
TNFα -1031	F-TATGTGATGGACTCACCAGGT R-CCTCTACATGGCCCTGTCTT	55	264
TNFα -863	F-GGCTCTGAGGAATGGGTTAC R-CTACATGGCCCTGTCTTCGTTACG	57	125
TNFα -857	F-GGCTCTGAGGAATGGGTTAC R-CCTCTACATGGCCCTGTCTAC	56	128
TNFα -376	F-CCCCGTTTTCTCTCCCTCAA R-TGTGGTCTGTTTCCTTCTAA	52	105
TNFα -308	F-GAGGCAATAGGTTTTGAGGGCCAT R-GGGACACACAAGCATCAAG	55	147
TNFα - 238	F-AAACAGACCACAGACCTGGTC R- CTCACACTCCCCATCCTCCCGGATC	64	309

— IL-1RN: 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°C using the following primers: 5'CTCAGCAACACTCCTAT3' (Forward) and 5'TCCTGGTCTGCAGGTAA3' (Reverse).

Electrophoresis was performed on 1.5% agarose gel stained by ethidium bromide to determine the size of amplified PCR products. The gels were photographed by using the gel documentation instrument. In addition, a 50 bp size marker was loaded onto the gel. The chi-square test was used to analyze differences in the TNF-α and IL-1RN genotype distribution and allele frequency between the case and control groups (SPSS software version 20). The odds ratio (OR) was used as a measure of the strength of the association between allele frequencies and RPL. All P values were two-tailed and 95% confidence intervals (CI) were calculated. P values < 0.05 were considered statistically significant.

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RESULTS

100 patients with at least three unexplained RPL (mean 5, range 3–7) and 100 age and ethnically matched controls who had at least two successful delivery from Iranian Azeri Turkish origin were screened for the TNF-α (1031T/C, 863C/A, 857C/T, 376G/A, 308G/A, and 238G/A) and IL-1RN polymorphisms. The results were as follows:

TNF-α allele frequencies and genotypes distribution in RPL patients and control subjects are shown in (Tables 2 and 3), respectively. With exception of -857 C/T variant, there were no significant association between the genotype prevalence of TNF-α polymorphisms in the case and the control groups. According to TNF-α -857 variant, there was a lower frequency of CC genotype in RPL patients than that of controls which was statistically significant (45% vs. 63%, OR = 0.48, CI = 0.26–0.87, p = 0.01). In contrast, the frequency of CT genotype across the cases was significantly higher than controls (47% vs. 30%, OR = 2.06, CI = 1.11–3.86, p = 0.02). However, no significant differences were displayed for TNF-α -857T (mutant) allele (31.5% vs. 22%, OR = 1.63, CI = 1.01–2.61, p = 0.42) and homozygous (TT) genotype (8% vs.7%, OR = 1.15, CI = 0.36–3.78, p = 1.00) among the case and control groups, respectively. Hence, it seems that being heterozygous for TNF-α -857 variant may lead to an increase in susceptibility to the RPL.

In IL-1RN the sizes of amplified alleles were 410 bp, 240bp, 500bp, 325bp and 595 bp, respectively. No statistically significant difference was 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 prevalence was not significantly different in the RPL patients from the healthy subjects (Table 4). The genotype and allele frequencies of women experiencing RPL and their healthy controls and the associated ORs are shown in Table 5 and Table 6. As can be found from the table, there is no meaningful differences among the prevalence of IL-1RN alleles in the control group compared to cases. 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;

Table 2. Restriction fragment length polymorphism (RFLP) analysis

Polymorphism	Size of amplified fragment	Restriction enzyme	Genotype	Size of digested fragments
-1031 T/C	264	BbsI	TT	264
			TC	264-193-71
			CC	193-71
-863 C/A	125	TaiI	CC	125
			CA	125-104-21
			AA	104-21
-857 C/T	128	TaiI	CC	109-19
			CT	128-109-19
			TT	128
-376 G/A	105	Tsp5091	GG	105
			GA	105-83-22
			AA	83-22
-308 G/A	146	NcoI	GG	125-21
			GA	146-125-21
			AA	146
-238 G/A	154	BamHI	GG	129-25
			GA	154-129-25
			AA	154

Table 3. Distribution of TNF-α genotypes among cases and control subjects

Polymorphism	Genotype	RPL Cases (100)	Controls (100)	P value	OR
-1031 T/C	TT	71	63	0.29	1.43
	TC	27	33	0.44	0.75
	CC	2	4	0.68	0.79
-863 C/A	CC	72	68	0.64	1.21
	CA	26	27	1.0	0.95
	AA	2	5	0.44	0.38
-857 C/T	CC	45	63	0.01	0.48
	CT	47	30	0.02	2.06
	TT	8	7	1.00	1.15
-376 G/A	GG	99	98	1.00	2.02
	GA	1	2	1.00	0.49
	AA	0	0	1.00	-
-308 G/A	GG	4	9	0.25	0.42
	GA	84	86	0.84	0.85
	AA	12	5	0.12	2.59
-238 G/A	GG	96	97	1.00	0.74
	GA	4	2	0.68	2.04
	AA	0	1	1.00	0.00

OR: 1/969–0/789). On the other side, no IL1RN*5 was found and IL1RN*4 allelic frequency was only 0.5% in both groups.

DISCUSSION

According to the heterogeneous entity of pregnancy loss, different factors have been considered to be related with this unpleasant event including coagulation factor gene polymorphisms [19], HLA-G polymorphisms [20], anatomical problems, chromosomal abnormality [3] and some other reasons [6]. Several projects have involvement in Iran abroad polymorphisms TNF and IL-1 receptor antagonist gene was performed in recurrent pregnancy loss. Some of these studies of polymorphisms as a risk factor for recurrent pregnancy loss have been introduced, while some relationship between this polymorphism and recurrent miscarriage have been denied.

Table 4. TNF-α polymorphisms allele frequencies among cases and control subjects

Polymorphism	Minor Allele	Cases (%)	Controls (%)	P-value	OR (95% CI)
-1031 T/C	C	15/5	20/5	0/24	0/71 (0/41–1/22)
-863 C/A	A	15	18/5	0/42	0/77 (0/44–1/36)
-857 C/T	T	31/5	22	0/42	1/63 (1/01–2/61)
-376 G/A	A	0/5	1	1/00	0/49 (0/01–7/04)
-308 G/A	A	54	48	0/27	1/27 (0/84–1/92)
-238 G/A	A	2	2	1/00	1/00 (0/20–4/82)

Table 5. Genotype frequencies of the IL-1RN polymorphism among Iranian RPL patients and healthy fertile women

Genotype IL-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 6. Allelic frequencies of the IL-1RN polymorphisms among Iranian RPL patients and healthy fertile women

Allele IL-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	–

It has been shown that Th1 cells play a crucial role in an inflammatory response and through the production of pro-inflammatory cytokines, IL-1, IFN-γ and TNF-α; they will affect the pregnancy outcome. TNF-α levels are also elevated in both mother and her embryo which is related with preterm parturition [21]. Considering the vital effect of TNF-α on pregnancy outcome and according to the varied reports on the association of different polymorphisms and the occurrence of RPL, we focused on six TNF-α gene polymorphisms. Zhang et al. also found that the -238G/A polymorphism might be related with the risk of recurrent spontaneous abortion [22]. However, there are some studies in which TNF-α -308 has been known to be associated with pregnancy loss [1]. In a study among Iranian women, no significant association between TNF-α -308 and RPL was observed [23]. TNF-α –1031T/C, –863C/A, –857C/T, –376G/A, –308G/A, –238G/A, and +488G/A single nucleotide polymorphisms were investigated across Bahraini Arabs by Finnan et al. Among these variants, –1031T/C, –376G/A, and –238G/A were concluded to be independently associated with recurrent miscarriage [24]. Based on another report, TNF-α -863 variant was not also related to RPL in Caucasian women [13]. Babbage et al. conducted an investigation into the predisposing effect of TNF-α, IFN-γ and IL-10 polymorphisms on the occurrence of RPL and no relation were found [25]. Since various ethnic groups were selected in different studies, for instance, Caucasian [22], Iranian [23], Indian [8] or Arabs [24]; it is not illogical to relate the differences in the results of these studies to the effect of different ethnicity of study population. Besides, sample size, selected cases and selection criteria [26], geographic differences [19] and the other involved genes [27] might be attributed to the conflicting results. Apart from TNF-α -857 variant, our results were in concordance with previously mentioned studies. In this regard, it was found that TNF-α -857 CT genotype was highly associated with RPL in Iranian Azeri Turkish women, while no significant association was detected for other five polymorphisms. Thereby, it is suggested that TNF-α -857 C/C variant might have a protective effect against RPL, but the -857 C/T variant is probably a genetic risk factor for the occurrence of RPL. Therefore, it is suggested that for the future studies, the effect of -857C/T polymorphism on the predisposition to RPL should be considered with more attention. Finally, in the present study, an association was found just for TNF-α -857 gene polymorphism and no remarkable differences were observed for the other TNF-α variants when compared to normal controls.

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. Karthukorpi et al, reported that the frequency differences of IL-1RN*1 and -1RN*2 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 [28]. According to IL-1RN*1 and IL-1RN*2, the results of our investigation were in concordance with Karthukorpi, but it did not confirm 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 were not meaningful (P: 0.16). Such a result was obtained by Die et al., who stated that IL-1RN*2 is not related to idiopathic recurrent spontaneous abortion in the Chinese Han population [29]. Vargas-Alarcon et al. compared the prevalence of IL-1RN, IL-6, IL-10, INF-γ, and TNF-α gene polymorphisms and found a significantly different distribution of these in the Mexican population [30]. Linjawi et al., compared 206 women with recurrent miscarriage with their controls in terms of IL-1RN*2 alleles and found no noticeable differences between their frequencies [17]. Similar to Linjawi, Agrawal [31] and Traina [26] studies as well as Levrant et al. [15], 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. [32]. In contrast, Perni et al. explained an increase in spontaneous abortions in the presence of fetal IL-1RN*1 [33]. As has been mentioned in the literature, the controversial reports from different studies can be satisfied by various reasons: the differences in the selected study groups [15], divergent sample sizes [31], accidental events, other involved genes and the mechanisms regulating the production of such cytokines [27] and the different environmental factors of the two groups [34]. It is believed that finding the association of gene polymorphisms and unexplained abortions will provide us a better understanding about patients' problem or determination of women who are at the risk of pregnancy loss. Furthermore, identification of gene variants would change the treatment strategy of the subjects [15].

CONCLUSIONS

In conclusion, the exact role of IL-1RN polymorphisms in RPL is not still fully understood. Thus, 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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Conflicts of interest

The authors declared that they had no conflicts of interest.

REFERENCES

1. Ramírez YL, Vivenes M, Miller A, et al. Prevalence of the coagulation factor XIII polymorphism Val34Leu in women with recurrent miscarriage. Clinica Chimica Acta. 2006; 374(1-2): 69–74, doi: 10.1016/j.cca.2006.05.032.
2. Omwandho CA, Tinneberg HR, Tumbo-Oeri AG, et al. Recurrent pregnancy losses and the role of immunotherapy. Arch Gynecol Obstet. 2000; 264(1): 3–12, doi: 10.1007/pl00007480, indexed in Pubmed: 10985611.
3. Calderwood CJ. Thromboembolism and thrombophilia in pregnancy. Current Obstetrics & Gynaecology. 2006; 16(6): 321–6.
4. Hajizadeh YS, Emami E, Nottagh M, et al. Effects of interleukin-1 receptor antagonist (IL-1Ra) gene 86 bp VNTR polymorphism on recurrent pregnancy loss: a case-control study. Horm Mol Biol Clin Investig. 2017; 30(3), doi: 10.1515/hmbci-2017-0010, indexed in Pubmed: 28593919.
5. Isazadeh A, Haj Az, Tariverdi N, et al. Effects of coagulation factor XIII (Val-34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. LaboratoriumsMedizin-Journal of Laboratory Medicine. 2017; 41(2): 89–92.
6. Hefler LA, Tempfer CB, Bashford MT, et al. A polymorphism of the interleukin-1beta gene and idiopathic recurrent miscarriage. Fertil Steril. 2001; 76(2): 377–379, doi: 10.1016/s0015-0282(01)01914-8, indexed in Pubmed: 11476790.
7. Baxter N, Sumiya M, Cheng S, et al. Recurrent miscarriage and variant alleles of mannose binding lectin, tumour necrosis factor and lymphotoxin alpha genes. Clin Exp Immunol. 2001; 126(3): 529–534, doi: 10.1046/j.1365-2249.2001.01663.x, indexed in Pubmed: 11737072.
8. Gupta R, Prakash S, Parveen F, et al. Association of CTLA-4 and TNF-α polymorphism with recurrent miscarriage among North Indian women. Cytokine. 2012; 60(2): 456–462, doi: 10.1016/j.cyto.2012.05.018, indexed in Pubmed: 22727980.
9. Brogin Moreli J, Cirino Ruocco AM, Vernini JM, et al. Interleukin 10 and tumor necrosis factor-alpha in pregnancy: aspects of interest in clinical obstetrics. ISRN Obstet Gynecol. 2012; 2012: 230742, doi: 10.5402/2012/230742, indexed in Pubmed: 22462002.
10. Rossi M, Sharkey AM, Viganò P, et al. Identification of genes regulated by interleukin-1beta in human endometrial stromal cells. Reproduction. 2005; 130(5): 721–729, doi: 10.1530/rep.1.00688, indexed in Pubmed: 16264101.
11. Glueck CJ, Wang P, Bornovali S, et al. Polycystic ovary syndrome, the G1691A factor V Leiden mutation, and plasminogen activator inhibitor activity: associations with recurrent pregnancy loss. Metabolism. 2003; 52(12): 1627–1632, doi: 10.1016/j.metabol.2003.06.001, indexed in Pubmed: 14669168.
12. Costeas PA, Koumouli A, Giantsiou-Kyriakou A, et al. Th2/Th3 cytokine genotypes are associated with pregnancy loss. Hum Immunol. 2004; 65(2): 135–141, doi: 10.1016/j.humimm.2003.11.007, indexed in Pubmed: 14969768.
13. Pietrowski D, Bettendorf H, Keck C, et al. Lack of association of TNFalpha gene polymorphisms and recurrent pregnancy loss in Caucasian women. J Reprod Immunol. 2004; 61(1): 51–58, doi: 10.1016/j.jri.2003.12.001, indexed in Pubmed: 15027477.
14. Zhou L, Cai J, Liu G, et al. Associations between interleukin-1 gene polymorphisms and coronary heart disease risk: a meta-analysis. PLoS One. 2012; 7(9): e45641, doi: 10.1371/journal.pone.0045641, indexed in Pubmed: 23029154.
15. Levrant S, Coulam C, Jeyendran R. ORIGINAL ARTICLE: Interleukin 1 Receptor Antagonist Gene Polymorphisms Are Not Risk Factors for Recurrent Pregnancy Loss: Evaluation of Couples. American Journal of Reproductive Immunology. 2008; 60(3): 224–228, doi: 10.1111/j.1600-0897.2008.00617.x.
16. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis. 2002; 34(2): 204–209, doi: 10.1086/338261, indexed in Pubmed: 11740709.
17. Linjawi S, Li TC, Laird S, et al. Interleukin-1 receptor antagonist and interleukin-1 beta polymorphisms in women with recurrent miscarriage. Fertil Steril. 2005; 83(5): 1549–1552, doi: 10.1016/j.fertnstert.2004.10.052, indexed in Pubmed: 15866604.
18. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 2009; 27: 519–550, doi: 10.1146/annurev.immunol.021908.132612, indexed in Pubmed: 19302047.
19. Isazadeh A, Haj Az, Tariverdi N, et al. Effects of coagulation factor XIII (Val-34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. LaboratoriumsMedizin-Journal of Laboratory Medicine. 2017; 41(2): 89–92.
20. Jassem RM, Shani WS, Loisel DA, et al. HLA-G polymorphisms and soluble HLA-G protein levels in women with recurrent pregnancy loss from Basrah province in Iraq. Hum Immunol. 2012; 73(8): 811–817, doi: 10.1016/j.humimm.2012.05.009, indexed in Pubmed: 22651916.
21. Moura E, Mattar R, de Souza E, et al. Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. J Reprod Immunol. 2009; 80(1-2): 115–121, doi: 10.1016/j.jri.2008.11.007, indexed in Pubmed: 19375805.
22. Zhang B, Liu T, Wang Z. Association of tumor necrosis factor-α gene promoter polymorphisms (-308G/A, -238G/A) with recurrent spontaneous abortion: a meta-analysis. Hum Immunol. 2012; 73(5): 574–579, doi: 10.1016/j.humimm.2012.01.008, indexed in Pubmed: 22369788.
23. Kamali-Sarvestani E, Zolghadri J, Gharesi-Fard B, et al. Cytokine gene polymorphisms and susceptibility to recurrent pregnancy loss in Iranian women. J Reprod Immunol. 2005; 65(2): 171–178, doi: 10.1016/j.jri.2005.01.008, indexed in Pubmed: 15811521.
24. Finan RR, Al-Irhayim Z, Mustafa FE, et al. Tumor necrosis factor-alpha polymorphisms in women with idiopathic recurrent miscarriage. J Reprod Immunol. 2010; 84(2): 186–192, doi: 10.1016/j.jri.2009.12.005, indexed in Pubmed: 20106534.
25. Babbage SJ, Arkwright PD, Vince GS, et al. Cytokine promoter gene polymorphisms and idiopathic recurrent pregnancy loss. J Reprod Immunol. 2001; 51(1): 21–27, indexed in Pubmed: 11438378.
26. Traina E, Daher S, Moron AF, et al. Polymorphisms in VEGF, progesterone receptor and IL-1 receptor genes in women with recurrent spontaneous abortion. J Reprod Immunol. 2011; 88(1): 53–57, doi: 10.1016/j.jri.2010.07.006, indexed in Pubmed: 20956022.
27. Unfried G, Tempfer C, Schneeberger C, et al. Interleukin 1 receptor antagonist polymorphism in women with idiopathic recurrent miscarriage. Fertil Steril. 2001; 75(4): 683–687, indexed in Pubmed: 11287019.
28. Karhukorpi J, Laitinen T, Kivelä H, et al. IL-1 receptor antagonist gene polymorphism in recurrent spontaneous abortion. J Reprod Immunol. 2003; 58(1): 61–67, doi: 10.1016/s0165-0378(02)00044-x, indexed in Pubmed: 12609525.
29. Dai MZ, Pan YQ, Xu DP, et al. IL-1 receptor antagonist gene polymorphism in idiopathic recurrent spontaneous abortion in a Chinese Han population. Int J Immunogenet. 2010; 37(5): 393–396, doi: 10.1111/j.1744-313X.2010.00939.x, indexed in Pubmed: 21182747.
30. Vargas-Alarcon G, Ramírez-Bello J, Juárez-Cedillo T, et al. Distribution of the IL-1RN, IL-6, IL-10, INF-γ, and TNF-α Gene Polymorphisms in the Mexican Population. Genet Test Mol Biomarkers. 2012; 16(10): 1246–1253, doi: 10.1089/gtmb.2012.0100, indexed in Pubmed: 22971140.
31. Agrawal S, Parveen F, Faridi RM, et al. Interleukin-1 gene cluster variants and recurrent pregnancy loss among North Indian women: retrospective study and meta-analysis. Reprod Biomed Online. 2012; 24(3): 342–351, doi: 10.1016/j.rbmo.2011.11.021, indexed in Pubmed: 22285242.
32. Jaaskelainen E, Keski-Nisula L, Toivonen S, et al. Polymorphism of the interleukin 1 receptor antagonist (IL1Ra) gene and placental abruption. J Reprod Immunol. 2008; 79(1): 58–62, doi: 10.1016/j.jri.2008.03.003, indexed in Pubmed: 18423886.
33. Perni SC, Vardhana S, Tuttle SL, et al. Fetal interleukin-1 receptor antagonist gene polymorphism, intra-amniotic interleukin-1beta levels, and history of spontaneous abortion. Am J Obstet Gynecol. 2004; 191(4): 1318–1323, doi: 10.1016/j.ajog.2004.05.071, indexed in Pubmed: 15507960.
34. Barton PT, Gerber S, Skupski DW, et al. Interleukin-1 receptor antagonist gene polymorphism, vaginal interleukin-1 receptor antagonist concentrations, and vaginal ureaplasma urealyticum colonization in pregnant women. Infect Immun. 2003; 71(1): 271–274, doi: 10.1128/iai.71.1.271-274.2003, indexed in Pubmed: 12496176.