

# IL-6, IL-10 and IL-17 Gene Polymorphisms in Iranian Women with Recurrent Miscarriage

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## ABSTRACT

**Background:** Pro-inflammatory and anti-inflammatory cytokines and polymorphisms of their genes have been described to be involved in the pathogenesis of recurrent miscarriage (RM). **Objective:** To investigate the association between RM and five polymorphisms of cytokine genes, interleukin 10 (IL-10), (-592 A/C, -819 C/T, -1082 A/G), IL-6 (-174 C/G) and IL-17 (-197 G/A) in Iranian women. **Method:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to determine the frequencies of the IL-6, IL-10 and IL-17 gene polymorphisms in 85 women with RM compared with 104 healthy controls. **Results:** The frequencies of IL-10 promoter gene polymorphisms (-592 A/C and -819 C/T) were significantly higher in RM women than those in controls ( $p=0.003$ ). However, no statistically significant differences were observed in the frequencies of IL-6 (-174 C/G), IL-10 (-1082 A/G) and IL-17 (-197 G/A) polymorphisms between RM women and controls. **Conclusion:** These results suggest that IL-10 gene polymorphism screening might have some relevance in patients with RM, a suggestion which requires further studies.

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**Keywords:** Cytokine, Gene Polymorphism, PCR-RFLP, Recurrent Miscarriage

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## INTRODUCTION

Recurrent miscarriage (1) is defined as three or more consecutive pregnancy losses before the 20<sup>th</sup> week of gestation (2). The exact pathophysiology of RM is still unclear. However, several etiological factors such as chromosomal abnormality, anatomic defects, hormonal problems, thrombophilic disorders, infections and immune system factors have been proposed to contribute to RM (3,4). After encountering the antigen, T helper (Th) precursors differentiate into functionally T-cell lineages, including Th1 and Th2 cells, with unique patterns of cytokine production (5). Th1 derived cytokines like IL-2, Interferon gamma (IFN- $\gamma$ ) and Tumor necrosis factor alpha (TNF- $\alpha$ ) cause the pro-inflammatory effects involved in cell-mediated responses and delayed-type hypersensitivity reactions, whereas Th2 cells produce anti-inflammatory cytokines such as IL-4 and IL-10 (5,6). It is evident that during a normal pregnancy an adequate balance of pro-inflammatory and anti-inflammatory cytokines is critical to the success of pregnancy (6,7). Though higher levels of pro-inflammatory cytokines such as TNF- $\alpha$  (8), IFN- $\gamma$  (9), IL-1 $\beta$  (10), IL-6 (11) and IL-17 (12) have been shown to terminate pregnancy, anti-inflammatory cytokines such as IL-10 and TNF- $\beta$  are considered essential for the maintenance of pregnancy (13).

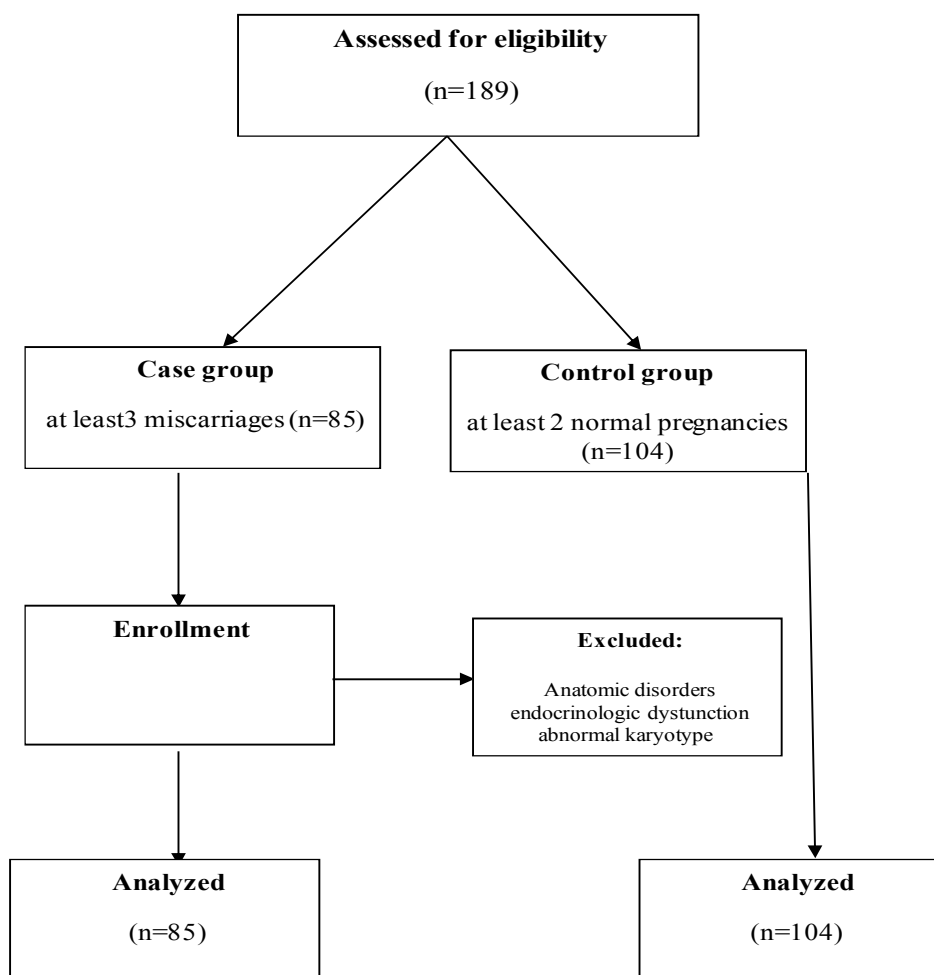
IL-6 is a multifunctional cytokine which is implicated in the inflammatory responses and T cell differentiation. IL-6, that is widely present in the female reproductive tract and gestational tissues, has particularly important functions in embryo implantation and placental development (14). A common C/G polymorphism located within the IL-6 gene promoter at nucleotide position 174 bp, upstream from the start site of transcription, has been reported to affect IL-6 expression and may contribute to miscarriage (15). However the predictive value of IL-6 for pregnancy outcome remains unclear.

IL-10 is an important immune suppressive and anti-inflammatory molecule that inhibits T cell proliferation and suppresses selectively Th1-mediated cellular responses (16). This cytokine is located on human chromosome 1 (1q31-q32) and plays an important role in the Th2 dependent immune responses. IL-10 gene polymorphisms have been reported to influence the level of cytokine production and implicated in RM pathogenesis (1,6,17,18).

Finally, a pro-inflammatory cytokine IL-17 (19) which is predominantly produced by a distinct subset of T helper cells (Th17) (20), is considered as a critical factor in inflammation, and autoimmunity (21) and may take a part in the pathogenesis of RM. Considering the potential role of cytokines and cytokine polymorphisms in RM patients, the aim of the present study was to investigate the association of RM with polymorphisms of IL-10 (-592 A/C, -819 C/T and -1082 A/G), IL-6 (-174 C/G) and IL-17 (-197 G/A) genes in Iranian RM compared to normal women.

## MATERIALS AND METHODS

**Subjects.** Eighty five women aged 27-44 years with a history of at least three miscarriages who referred to Avicenna Infertility Clinic, between April 2010 and March 2011 were recruited as RM group. Anatomic disorders, endocrinologic dysfunctions and abnormal karyotypes were considered as exclusion criteria. The flow chart for the enrolment of the patients and controls is presented in Figure 1.



**Figure 1.** Flow chart for the enrolment of the patients and controls in the study.

The control group consisted of 104 healthy women, aged 27-42 with at least two normal pregnancies and no pregnancy complications such as miscarriages, still births, small for gestational age fetuses, and preeclampsia. The study was approved by the Ethics Committee for Medical Research of Avicenna Research Institute and written consent was obtained from all participants.

**Blood Sample Collection and PCR Amplification.** Peripheral blood samples were collected under sterile conditions into tubes containing EDTA, then genomic DNA was extracted by standard methods, as described previously (22).

**Genotype Analysis.** Genomic DNA was amplified by polymerase chain reaction (PCR) using IL-10 (-1082 A/G, -819 C/T and -592 A/C), IL-6(-174 C/G) and IL-17 (-197 G/A) specific primers (Table 1). PCR amplifications were then confirmed by electrophoresis on 1.5% ethidium bromide-stained agarose gels.

Restriction enzyme digestions with Rsa I, Mea III, MnlI, (Roche Diagnostics, Mannheim, Germany) XmnI and NlaIII (Fermentas, EU) enzymes were employed to determine the IL-10 (-592 A/C, -819 C/T and 1082 A/G), IL-17 (-197 G/A) and IL-6 (-

174 C/G) variants, respectively (Table 1) and the resulting fragments were analyzed on 2.5-3.5% agarose gels.

**Statistical Analysis.** All the statistical analyses were performed using the statistical package for the social sciences (SPSS) software version 13.0 for Microsoft windows. Genotype distributions of cytokine gene polymorphisms were compared between cases and controls by Chi-square test. Correlations between the polymorphisms were calculated by spearman test.

**Table 1. Primers and restriction enzymes used for cytokine genes polymorphisms.**

Polymorphisms	Primers	Amplicon size (bp)	Restriction Enzyme	RFLP Size (bp)
IL-6 -174 C/G	F: 5'- TGA <del>C</del> TTCA <del>G</del> CTTTACTCTTTGT -3' R: 5'- CTGATTGGAAACCTTATTAAG -3'	198bp	Nla III	140, 58
IL-10 -592 A/C	F: 5'- GGTGAGCACTACCTGACTAGC -3' R: 5'- CCTAGGTCACAGTGACGTGG -3'	412 bp	Rsa I	236, 176
IL-10 -819 C/T	F: 5'- TCATTCTATGTGCTGGAGATG -3' R: 5'- TGGGGGAAGTGGGTAAGAGT -3'	209 bp	Mae III	125, 84
IL-10 -1082 A/G	F: 5'- CTCGCCGCAACCCAACTGGC -3' R: 5'- TCTTACCTATCCCTACTTCC -3'	137 bp	MnII	23 , 114
IL-17 -197 G/A	F: 5'- CAGAAGACCTACATGTTACT -3' R: 5'- GTAGCGCTATCGTCTCTCT -3'	344 bp	XmnI	213, 131

## RESULTS

A total of 85 women with RM and 104 normal controls were analyzed for carrying IL-6-174 C/G, IL-17 -197 G/A and three polymorphisms of IL-10 (-592 A/C, -819 C/T and -1082 A/G). There were no significant differences in the frequencies of the IL-10 (-1082 A/G,  $p=0.975$ ), IL-6 (-174 C/G,  $p=0.917$ ) and IL-17 (-197 G/A,  $p=0.276$ ) polymorphisms between the case and the control groups. However, IL-10 -592 A/C and -819 C/T gene polymorphisms showed significant differences between the two groups ( $p<0.05$ ) (Table 2).

The allele frequencies of the latter IL-10 gene polymorphisms were also calculated. The frequencies of A allele of IL-10 gene at position -592 were found to be 21.03% and 44.71% for RM women and controls, respectively. In addition the C allele frequencies IL-10 gene at position -819 were respectively 31.25% and 36.53% for RM women and controls, respectively.

Correlations between IL-10 -592 A/C and IL-10 -819 C/T ( $r=0.499$ ,  $p<0.001$ ), IL-10 -592 A/C and IL-10 -1082 A/G ( $r=0.187$ ,  $p=0.028$ ), IL-6 -174 C/G and IL-17 (-197G/A) ( $r=-0.178$ ,  $p=0.037$ ) gene polymorphisms were also detected by spearman correction test.

**Table 2. Prevalence of the five polymorphisms of cytokine genes in case and control groups and evaluation of their association with RM.**

Polymorphisms	Normal		Heterozygote		Homozygote		p-value
	Case (%)	Control (%)	Case (%)	Control (%)	Case (%)	Control (%)	
IL-6 -174 C/G	7.1	8.7	50.6	49	42.4	42.3	0.917
IL-10 -592 A/C	9.4	21.2	40	47.1	50.6	31.7	0.013*
IL-10 -819 C/T	8.2	13.5	60	75.7	31.8	10.8	0.006*
IL-10 -1082 A/G	41.2	40.4	38.8	40.4	20	19.2	0.975
IL-17 -197 G/A	48.2	38.1	43.5	55.2	8.2	6.7	0.276

\*significant

## DISCUSSION

Several lines of evidence suggest that cytokines are implicated in pathogenesis of unexplained RM (16). Relations between cytokine profiles and susceptibility to some diseases have also been described (23). In addition, cytokines, as immune regulatory molecules, have been addressed to have important and critical roles in reproductive events (18,24-27). It is generally accepted that a successful pregnancy is dependent on a shift away from Th1-type and a bias towards Th2-type immune responses (28). Previous studies have shown that maintenance of fetal survival has been associated with a Th2-type dominant response whereas a Th1-type response has been related to pregnancy failure (29). Cytokine production levels are partly under genetic control, and their gene expression can be changed by nucleotide variation (16).

Here, we attempted to investigate the association between polymorphisms of two pro-inflammatory (IL-6 and IL-17) and an anti-inflammatory (IL-10) cytokine genes with RM in Iranian women. Our results demonstrated an association for IL-10 gene polymorphisms including IL-10-592 A/C (rs1800872) and IL-10-819 C/T (rs1800871) but not IL-10-1082 A/G (rs1800896) promoter polymorphisms in idiopathic RM. Lack of any association between IL-10-1082 A/G polymorphism and RM was consistent with previous studies (6,18,30).

The significantly higher frequency of the IL-10 -592 A/C genotype in RM women than controls ( $p < 0.05$ ), that reported in this study, is in agreement with Kamali-Sarvestani *et al.* observation who reported a significant association between the presence of this polymorphism and the occurrence of RM in Iranian women (63% in women with RM and 46% in controls) (18). Zammiti *et al.* also demonstrated that IL-10-592 A/C (OR= 3.32) single nucleotide polymorphism (SNP) correlated with exclusively early (5-10 weeks) RM (6). Moreover, these findings are further supported by a case-control retrospective study on 350 Tunisian women (6). Statistical analysis of our results revealed that IL-10 -819 C/T polymorphism was also associated with RM ( $p < 0.05$ ). However, our findings are in contrast with reports by Prigoshin (7) and Kaur (31) who reported no association between RM patients and controls concerning the IL-10 gene

polymorphisms. Collectively, these reports highlight the need to analyze the association between IL-10 gene polymorphisms and RM in different ethnic groups.

It is well documented that RM is associated with reduced IL-6 protein and mRNA expression in the midsecretory phase of human endometrium (32). In this regard, we found no association between C/G -174 polymorphism at in IL-6 gene, a phenomenon also observed by others (7,17). The contradiction in the involvement of IL-6 C/G -174 gene polymorphism and IL-6 protein level in RM may partially be defined by presence of other IL-6 gene polymorphisms or the presence of IL-6 protein expression regulatory mechanisms (33).

Interleukin-17 plays a critical role in the induction of inflammation, autoimmunity and immunological rejection of foreign tissues (20,34). An increased prevalence of IL-17+ cells has been detected in peripheral blood and decidua in unexplained RM women (35). However our data fall short of showing any significant association between IL-17 -197 G/A gene polymorphism (rs2275913) and RM. No statistically significant association between IL-17 polymorphism and RM may be related to the ethnic origin of the samples and thus needs to be confirmed by further investigations. In conclusion, our results demonstrated an association for IL-10 -592A/C and -819C/T gene polymorphisms with idiopathic RM which suggest that IL-10 gene polymorphism screening might have some relevance in RM patients. In this regards, additional research would help clarify the role of IL-10 gene polymorphisms in women with RM.

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