Investigating the Association of IL-17A and IL-17F with Susceptibility to Pre-eclampsia in Iranian Women

Fahimah Anvari[1,2], Feryal Dabagh-Gorjani[1,2], Mohammad-Sadegh Soltani-Zangbar[1,2], Eskandar Kamali-Sarvestani[1,3,4], Zahra Malek-Hosseini[1], Behrouz Gharesi-Fard[1,4,5*]

[1]Department of Immunolog y, School of Medicine, [2]Student Research Center, [3]Autoimmune Diseases Research Center, [4]Proteomics Laboratory, School of Advanced Medical Sciences and Technologies, [5]Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Background: Pre-eclampsia (PE) is one of the most important and life-threatening pregnancy disorders that affect at least 3-5% of all pregnancies. Imbalance in helper T cell functions may play a role in predisposing to PE or severity of the disease. Elevated frequencies of Th17 cells in the peripheral blood of PE patients have been reported. Several single nucleotide polymorphisms (SNP) within IL-17 gene have been identified that may affect the IL-17 production. **Objectives:** To investigate the association between IL-17A (-197A/G) and IL-17F (+7488T/C) gene polymorphisms and susceptibility to PE in a group of Iranian women. Moreover, to study any correlation of the polymorphisms data with the level of IL-17, at mRNA level in the paternal and maternal parts of the placentas and also at protein level in the peripheral and placental blood samples. **Methods:** A group of 261 PE patients and 278 age-matched healthy women with at least two previous normal pregnancies formed the cases and controls of this study. IL-17A (-197A/G) and IL-17F (+7488T/C) polymorphisms were genotyped using PCR-RFLP method. The protein level of IL-17A was assessed in the sera of 40 PE and 40 healthy women using ELISA method and mRNA expression was also measured in placental samples of 19 PE and 19 control women using Q-PCR technique. **Results:** Statistical analysis indicated that there were no differences in genotype, allele or haplotype frequencies regarding the studied SNPs between cases and controls. The level of IL-17A was elevated in the placental blood and the fetal tissue at protein and mRNA levels ($p< 0.009$ and $p<0.000$, respectively) in PE as compared with the healthy women. **Conclusions:** The effect of IL-17 cytokine in pre-eclampsia is not due to the studied cytokine polymorphisms but local production of IL-17 might have an effect on the predisposition to the disease.

Anvari F,et al. Iran J Immunol. 2015; 12(2):117-128

Keywords: IL-17, Pre-eclampsia, Polymorphism

---

*Corresponding author: Dr. Behrouz Gharesi-Fard, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Tel/Fax: (+) 98 711 2351575, e-mail: gharesifb@sums.ac.ir

INTRODUCTION

Pre-eclampsia (PE) is one of the most important and life-threatening pregnancy disorders that affect human pregnancies. PE is defined and diagnosed with the occurrence of hypertension along with proteinuria after 20 weeks of gestation (1). PE is a leading cause of maternal and fetal mortality and morbidity worldwide. While PE affects around 8% of the human pregnancies worldwide, the incidence of the disease may increase up to three times more depending on the geographic areas and ethnic or social factors (2). Interestingly, PE is reported to be involved in 12% of pre-termed neonate deliveries and 18.2% of maternal deaths in Iran (3). Till now the etiological factors that predispose women to PE are not well known, but there is no doubt that PE is a placentation disease (4). Indeed defect in the placenta formation which is characterized by widespread maternal endothelial dysfunction and reduction of uteroplacental blood flow is the main reason for PE development (5). Beside the role of placenta in PE formation, a group of etiological factors including immunological, environmental, and genetic factors may also play roles in PE development (6). Pre-eclampsia is more common among the family members of a pre-eclamptic women (7). Moreover, the prevalence of the disease is not equal among different ethnic groups (8). Immune system has a major role in a normal pregnancy and also plays an important role in the formation of the human placenta (9). Indeed, it seems that a fine balance between immune stimulation and immune regulation of the immune system support a normal pregnancy. T cells play a central role in activation and also regulation of the immune responses. Th17 subsets are a recently described subpopulation of TCD4+ cells with important roles in the protection against extracellular bacteria, stimulation of inflammatory responses and also autoimmune diseases (10). Systemic inflammation is a hallmark of preeclampsia and Th17 cells may be responsible for the inflammatory processes in the pregnancy period that cannot be explained by Th1/Th2/Treg responses. Th17 cells differentiate upon exposure to combinations of IL-1, IL-6 and transforming growth factor beta (TGF-β) and characterized by preferential synthesis of IL-17, and IL-22 (11). IL-17 family consists of six members including IL-17A (also previously known as IL-17) to IL-17F and five receptors (12). IL-17A and IL-17F show the highest amino acid sequence homology (about 50% more than any other members of the IL-17 family) and are the members of the IL-17 cytokine family responsible for the pathogenic activity of the Th17 cells (13). IL-17A and IL-17F genes are located on the same chromosome at position 6p12. IL-17A and IL-17F both bind to the same receptor and likely to have similar biological activities (14). Although little is known regarding the role of Th17 cells in pregnancy period or pregnancy related diseases, elevated prevalence of Th17 or Th1/Th17 cells in the third trimester of pregnancy has been reported (15). Moreover, increased frequency of Th17 cells in the peripheral blood of PE patients compared to healthy pregnant women in the third trimester of pregnancy have been reported (16). Several single nucleotide polymorphisms (SNP) within IL-17 gene have been identified that may affect the IL-17 production (17,18). Allelic variants within IL-17 genes might relate to the expression or activity of the cytokine and therefore susceptibility to or increase in the severity of PE (19). Two polymorphisms within IL-17A and IL-17F genes, IL-17A (rs2275913) and IL-17F (rs763780), have been reported to be associated with a group of autoimmune or inflammatory diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, and chronic fatigue

syndrome (17,20-22). Interestingly the relation between IL-17 gene polymorphisms and PE has not been investigated, yet.

The aim of the present study was to investigate the association between IL-17A (-197A/G) and IL-17F (+7488C/T) gene polymorphisms and susceptibility to and severity of PE in group of Iranian women. Since there was no data available about the IL-17A and IL-17F allele frequencies in Iranian population, we selected two IL-17A and IL17F polymorphisms based on the NCBI SNP database and reports with a minimum allele frequency of at least 10% among different populations. Moreover, to connect the polymorphisms data and the level of IL-17, we also measured IL-17A at mRNA level in the fetal and maternal parts of the placentas from preeclamptic and healthy women using real time PCR method and also at protein levels in the peripheral and placental blood samples using ELISA technique.

MATERIALS AND METHODS

Subjects. This case-control study was undertaken on 261 Iranian pregnant women (mean age 27.9) diagnosed with preeclampsia (130 mild and 131 severe form) and 278 healthy pregnant women (mean age 26.7) with at least two previous pregnancies without any complication. Written informed consent was obtained for using the placental and blood samples and the study protocol was approved by the local Ethics Committee of Shiraz University of Medical Sciences. Diagnosis of the disease and its severity was based on the level of proteinuria and hypertension. Women with more than 0.3 gr protein in a 24-hr urine specimen along with a minimum of two blood pressure readings greater than 140/90 mmHg were considered preeclamptic. Among preeclamptic women who had two blood pressure readings greater than 160/110 mmHg along with more than 5 gr proteinuria was diagnosed as severe preeclampsia. Women with the history of autoimmune or other immunological disorders (including infectious diseases, such as HIV) were excluded from the study.

Sampling. Two ml of anti-coagulated peripheral venous blood were collected from all cases and controls and used for genotyping. Genomic DNA was extracted from anti-coagulated peripheral blood using PrimePrep Genomic DNA Isolation Kit (from Blood) (GENET BIO, Korea), following the manufacturer's instructions and stored at -20°C. Moreover two ml more clot peripheral blood were collected from 40 out of 261 PE (24 mild and 16 severe form) and also 40 out of 278 healthy pregnant women before section to separate the serum for IL-17 assay by ELISA test. In addition, 2 ml of placental blood from 19 out of 40 PE (11 mild and 8 severe form) and 19 out of 40 healthy pregnant women were also collected and the serum was separated. All sera were stored after collection at -70°C until the time of analysis. Besides, the placentas samples from 15 preeclamptic (10 mild and 5 severe form) and also 15 healthy women were collected immediately after the cesarean section for the real time PCR tests. After section, the maternal parts of the placentas were separated from the fetal parts. Approximately, 500 mg of each tissue was collected, washed with cold normal saline, and stored in liquid nitrogen until extraction of total ribonucleic acid (RNA). Total RNA was isolated from placental tissue using Total RNA Purification Kit (Jena bioscience, Germany) according to the manufacturer's recommendations. The concentration of the extracted RNA was determined using a Nano drop instrument and stored at -70°C before synthesis of cDNA.

Genotyping. IL-17A -197A/G and IL-17F +7488T/C genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique using TECHNE, Thermal Cycler instrument (TECHNE, Version 3.1) and the following pairs of primer: forward primer: 5′- GCCAAGGAATCTGTGAGGAA-3′, reverse primer: 5′-TGCCTGCTATGAGATGGACA-3′; for rs2275913 (IL-17A -197A/G) and, forward primer: 5′ GTTCCCATCCAGCAAGAGAC-3′, reverse primer: 5′ AGCTGGGAATGCAAACAAAC-3′ for rs763780 (IL-17F +7488T/C) (18). For genotyping of IL-17A -197A/G SNP, a 424bp product was amplified and subjected to digestion using XagI restriction enzyme (Fermentas, Lithunia) to create 260 and 164 bp fragments (Figure 1A).

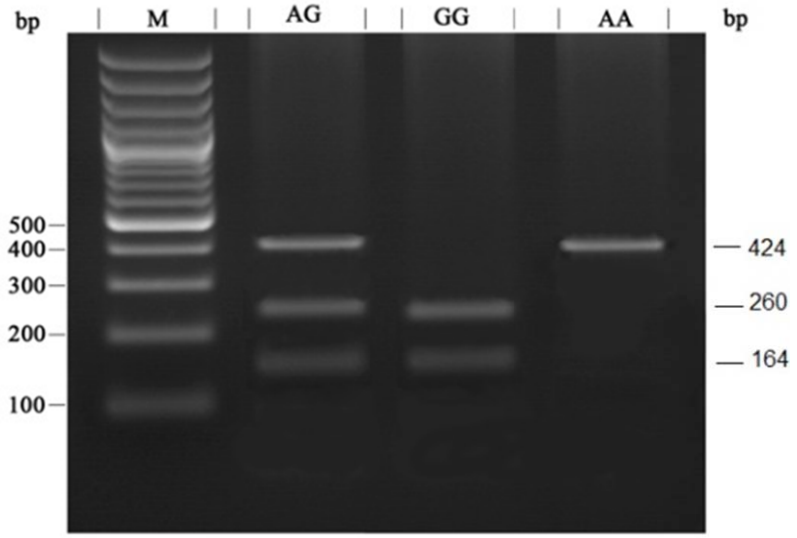

Figure 1. Typical example of a 3% agarose gel electrophoresis for identification of IL-17 polymorphisms at po sition -197A/G after digestion with XagI enzyme (A) a nd at position IL-17F (+7488T/C) after digestion with NlaII enzyme (B).

Figure 1A. M: DNA Marker, AG: Heterozygous genotype digested to three fragment (424bp, 260bp, 164bp), GG: Homozygous for G allele di gested to tw o fragments (260bp, 164bp), AA:Homozygous for A allele with no digestion.

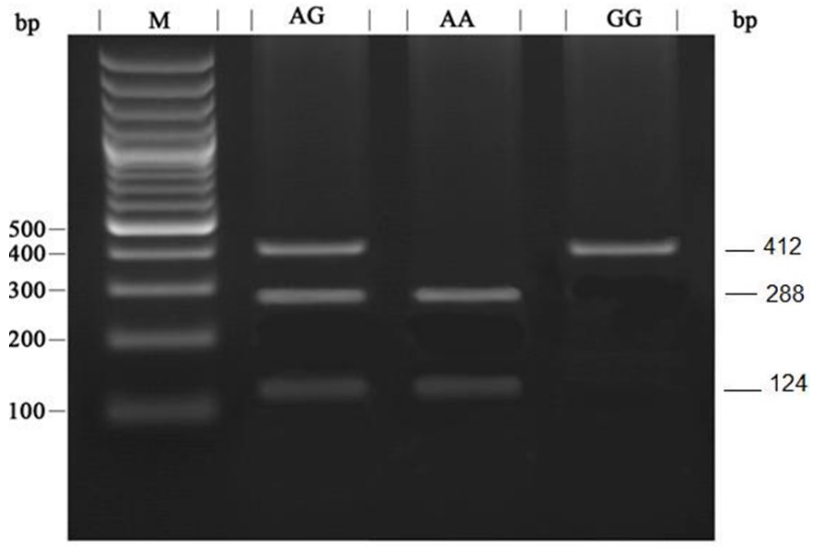

Figure 1B. M: DNA Marker, AG: Heterozygous genotype consisting of three fragments (412bp, 288bp, 124bp). AA: Homozygous for A allele digested to two fr agments (288bp, 124bp). GG: Homozygous for G allele with no digestion.

The amplified PCR product for IL-17F +7488T/C SNP was 412bp and after digestion with NlaII restriction enzyme (Fermentas, Lithunia) creates 124 and 288bp fragments (Figure 1B). The results of genotyping were monitored by electrophoresis in a 3% agarose gel.

ELISA. The concentrations of the IL-17A in peripheral blood of 40 PE patients and 40 healthy women and also in cord blood of 19 PE patients and 19 healthy controls were evaluated by enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits (Human IL-17A homodimer ELISA Ready-SET-Go USA). The assay was performed according to the manufacturer's instructions and the plates were read at 450 nm. The sensitivity of the kit was 4 pg⁄mL and all values below the sensitivity of the kits were considered as absent or zero.

Quantitative Real-Time PCR (Q-PCR). Reverse transcription reaction used to synthesis cDNA from total RNA using easy^{TM}cDNA synthesis kit (Pars Tous, Iran). Briefly, the mixture of oligo-dT, 700 ng RNA, and DEPC water was heated at 65°C for 5 minutes and was immediately transferred on ice. After adding the reverse transcriptase enzyme, cDNA was made and stored in aliquots at -70°C till performing the tests. Moreover the quality of the synthesized cDNA was checked by PCR method, using 18sRNA as housekeeping gene. For Q-PCR assay SYBR Green method (Ariatous, Iran) was used. Two µl of the synthesized cDNAs was used for amplification using IL-17A mRNA specific primers: forward 5′-GGAAGAAACAACGATGAC-3′, and reverse 5′-GATTCCTGCCTTCACTAT-3′ and 18S rRNA specific primers: forward 5′-CTCAACACGGGAAACCTCAC-3′ and reverse 5′-AAATCGCTCCACCAACTAAGAA-3 (23) as housekeeping control gene. Briefly, a mixture containing 20 picomol of each primer, 2 µl of target cDNA (selected after checking and normalization of C_T values), and 10 µl SYBR Green cocktail in a final volume of 20 µl were used for amplification. All assays were run in duplicates and the mean C_T was used for calculation. The amplification condition consisted of an initial denaturation at 95°C for 30 s followed by 40 cycles of denaturation at 95°C for 5 s and annealing and amplification at 60°C for 35 s. To quantify the gene expression, $2^{-\Delta\Delta C_T}$ methods was used. To minimize the assay variation and normalization the Q-PCR technique, calibrator sample and 18s rRNA were used in each run respectively.

Statistical Analysis. Comparisons between the distributions of the allele and genotype frequencies in cases and controls and different subgroups were performed using the chi-square test. Moreover Arlequin software was used for haplotype analysis. Independent student's *t-test* and Mann-Whitney test were used for quantification analysis of ELISA and Q-PCR tests. Statistical Package for the Social Sciences (SPSS) software, v. 16 (SPSS Inc, Chicago, IL, USA) was used for analysis and p values less than 0.05 were considered as statistically significant.

RESULTS

Genotype and Allele Distributions for IL-17A -197A/G and IL-17F +7488T/C SNPs. Figure 1 shows a typical electrophoresis results for IL-17A and IL-17F genotyping. The results of the statistical analysis for IL-17A -197A/G and IL-17F +7488T/C genotype and allele frequencies in PE and healthy women have been presented in Tables 1 and 2. Although the genotype frequencies in patients and healthy controls were in concurrence with Hardy-Weinberg equilibrium, no significant

differences in the distributions of allele or genotype frequencies between PE or the PE subgroups (Mild and Severe form) and healthy women were observed.

Table 1. Genotype and allele distributions for IL-17A -197A/G and IL-17F +7488T/C SNPs in the patients and healthy controls.

IL-17 SNPs	Allele	Controls Number (%)	Patients Number (%)	P Value	Genotype	Controls Number (%)	Patients Number (%)	P Value
IL-17A -197A/G	A	236 (42.4)	216 (41.4)	0.72	AA	24 (8.6)	21 (8.0)	0.86
					AG	188 (67.6)	173 (66.3)	
	G	320 (57.6)	306 (58.6)		GG	66 (23.8)	67 (25.7)	
IL-17F +7488T/C	T	515 (92.6)	468 (89.7)	0.11	TT	242 (87)	216 (82.8)	0.29
					TC	31 (11.2)	36 (13.8)	
	C	41 (7.4)	54 (10.3)		CC	5 (1.8)	9 (3.4)	

SNP: Single Nucleotide Polymorphism, p values calculated using chi-square test.

Table 2. Genotype and allele distributions for IL-17A -197A/G and IL-17F +7488T/C SNPs in severe and mild forms of PE.

IL-17 SNPs	Allele	Severe Number (%)	Mild Number (%)	P Value	Genotype	Severe Number (%)	Mild Number (%)	P Value
IL-17A -197A/G	A	102 (39.2)	114 (43.5)	0.32	AA	12 (9.2)	9 (6.9)	0.12
					AG	79 (60.3)	94 (72.3)	
	G	158 (60.8)	148 (56.5)		GG	40 (30.5)	27 (20.8)	
IL-17F +7488T/C	T	235 (90.4)	233 (88.9)	0.34	TT	111 (84.7)	105 (80.8)	0.23
					TC	14 (10.7)	22 (16.9)	
	C	25 (9.6)	29 (11.1)		CC	6 (4.6)	3 (2.3)	

SNP: Single Nucleotide Polymorphism, p values calculated using chi-square test.

Moreover, haplotype analysis also indicated that there were no differences between PE and healthy women regarding the haplotype frequencies for IL-17A -197A/G and IL-17F +7488T/C, SNPs (Table 3). However, linkage disequilibrium between A allele in IL-17A-197 and C allele in IL-17F+7488 positions (AC haplotype) in patients was observed.

As indicated in Tables 1-3, while the expected frequency for this haplotype is about 3.1%, the frequency of AC haplotype was 5.7% in PE women.

Table 3. Distributions of IL-17A -197A/G and IL-17F +7488T/C SNPs haplotypes in the patients and healthy controls.

IL-17A/ IL-17F haplotypes	Patients Number (%)	Controls Number (%)	P Value
AT	186 (35.6)	212 (38.1)	0.31
AC	30(5.7)	22 (4.0)	
GC	24(4.6)	18 (3.2)	
GT	282(54.1)	304 (54.7)	

P value calculated using chi-square test.

Comparison of IL-17A levels in the PE Patients and Healthy Controls. Evaluation of IL-17A concentration indicated that although the levels of this cytokine is not significantly different between PE patients and healthy women in peripheral blood samples, PE patients had elevated IL-17A levels compared with healthy women in their placental blood samples ($p=0.14$ and $p<0.009$, respectively, Table 4).

Table 4. Comparison of IL-17A levels in the PE patients and healthy controls.

Samples	Subjects	Number	Pg/ml Mean±SD	P Value
Peripheral blood	Patients	40	12 ± 6.7	0.14
	Controls	40	14.5 ±7.4	
Placental blood	Patients	19	48.7 ±36.2	<0.009
	Controls	19	23.2 ± 14.3	

P values calculated using independent samples *t-test.*

On the other hand, the levels of IL-17A were significantly elevated in the placental blood compared with the peripheral blood in both PE and healthy women ($p<0.001$ for both comparisons). Moreover, we analyzed the correlation of IL-17A levels in peripheral and placental blood samples, with disease form, genotype and allele frequencies of IL-17A. Results showed that there is no association between the levels of IL-17A in peripheral and placental blood samples and disease forms or genotype and allele frequencies (data have been not shown).

Comparison of IL-17A mRNA Expression in Different Parts of Placentas. To detect the concentration of IL-17A at mRNA level, we used placental tissue samples. Furthermore, we also checked the levels of this cytokine in maternal and fetal parts of the placental samples. Finally, the expression levels of IL-17A were compared between patients and healthy women as well as different forms of the disease using mean ΔCt and $2^{-\Delta\Delta C_T}$method.

Table 5. Comparison of IL-17A mRNA expression in different parts of placentas of the patients and healthy women.

Group	Samples	Mean ΔCt±SD	Fold Change	P Value
Patient	Fetal placenta	20.49 ± 0.76	1.72	0.000
	Maternal placenta	22.3 ± 0.61		
Controls	Fetal placenta	20.5 ± 0.44	1.34	0.000
	Maternal placenta	22.5 ± 0.50		

P values calculated using Mann-Whitney test.

Results indicated that although there are no overall significant differences between PE and healthy women regarding IL-17A expression at mRNA levels, but PE patients and healthy women expressed an increased levels of IL-17A mRNA in the fatal parts of the placenta in comparison with the maternal parts (Table 5 and Figure 2, P)

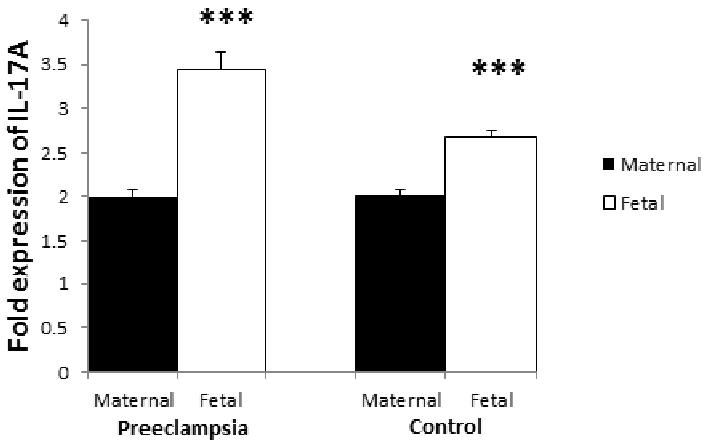

Figure 2. Comparison of the expression of IL-17A at mRNA level based on the fold expression in the different parts of placental tissue from the patients with preeclampsia and healthy control women.
PE=Preeclampsia, F=Fetal part of the placenta, M=Maternal part of the placenta. *** P<0.000, p values calculated using Mann-Whitney test.

DISCUSSION

Despite the numerous studies that have been published during the previous years, the exact etiological factors that predispose women to PE are not well known. Moreover, there is no predictive test for screen and diagnosis the PE women before manifestation of the clinical signs till now. Recent studies emphasized that PE might be an excessive inflammatory response of the mother immune system to the fetal tissues (24). The local

and generalized inflammation is thought to be associated with an imbalance of maternal Th1/Th2 and Th17/Treg responses to the fetus (25).

Moreover, different prevalence of the disease between different ethnic group and increased susceptibility to the disease among the family members of a PE women indicated that beside the immune responses, genetic factors also play an important role in predisposing to the disease. Therefore, based on the recent published data regarding the relation between inflammatory responses directed by Th17 lymphocytes and preeclampsia, in the present study we investigated the association between allelic polymorphisms within IL-17 gene, and IL-17 concentrations at mRNA and protein levels and predisposing to and severity of preeclampsia in a group of Iranian women. Both IL-17A and IL-17F are secreted by Th17 cells and are involved in the development of local tissue inflammation (26). Polymorphisms within genes might affect the production of cytokines. Regarding IL-17, the first studied polymorphism (rs2275913) is located at the 5′ promoter region of the IL-17A gene, which can affect IL-17A gene transcription (27). IL-17A -197A/G polymorphisms have been shown to be associated with a group of inflammatory diseases including ulcerative colitis, rheumatoid arthritis, and gastric carcinogenesis, but interestingly there is no report regarding the association between this polymorphism and preeclampsia (17,28). The second studied polymorphism (rs763780) causes a His-to-Arg substitution at amino acid 161 (H161R) and is located at the same chromosome (21). While there is no published paper regarding the association between IL-17F +7488T/C polymorphisms and preeclampsia, the association between this polymorphisms and gastro-duodenal ulcer diseases, chronic gastritis, gastric carcinogenesis and IBD have been reported (14,17,21,22,29). Moreover, a significant association between IL-17F +7488T/C polymorphisms and susceptibility to recurrent abortion in a group of Iranian women has been reported recently (30). As a first report, we observed that the C allele in IL-17F +7488T/C position showed increased frequencies in PE compared with the healthy women and GG genotype in IL-17A -197A/G position showed increased frequencies in patients with the severe form of the disease compared with the mild form (with a trend to significant p value ($p=0.11$ and $p=0.12$ respectively). However, the results of the present study failed to show statistically significant association between studied SNPs as well as haplotype distributions and susceptibility to or severity of PE. Interestingly, Najafi and coworkers recently reported an association between CC genotype in IL-17F +7488T/C position and susceptibility to recurrent miscarriage among Iranian women (30). Considering the number of samples in our study, further studies with a larger sample size is recommended to find probable associations between the studied polymorphisms and susceptibility to PE.

Regarding the relation between IL-17 levels and PE, there are controversial reports. While several authors have reported an increased level of IL-17 in preeclamptic women, others have reported unchanged or even decreased level of IL-17 in preeclamptic women (31,32). In the present study we measured the level of IL-17A at protein levels in peripheral and placental blood samples. Moreover, we tested maternal and fetal parts of the placental tissues to evaluate the IL-17A at mRNA level. Interestingly, while the mean level of IL-17A cytokines did not indicate a significant difference between PE and healthy women in the peripheral blood, the level of IL-17A showed an increased expression within placental blood of PE as compared with healthy women. These results indicated that the elevated level of IL-17A in the placental blood is more important in PE compare with the systemic level of IL-17A in the peripheral blood. In spite of

significant differences in IL-17A concentration between cases and controls in the placental blood, no statistically difference was found for IL-17A at the mRNA level. This discrepancy between protein and mRNA level might be due to the different samples that were used for each experiment. While the circulating or secretory IL-17A was assessed at protein level, tissue samples were used for mRNA assay. Indeed the detected IL-17A in placental blood is produced by both tissue and blood cells. These findings may illustrate the controversy in the published papers regarding the level of IL-17 cytokine in preeclampsia. Toldi*et al*. and Darmochwal-Kolarz and coworkers indicated that the percentage of IL-17 producing T lymphocytes was significantly higher in peripheral blood from preeclamptic than healthy women (33,34). In contrast, Ozkan*et al*. reported a decreased level of IL-17 in PE women compared with healthy normotensive women (35). The gestational week of the patients and methodology, especially using different samples, might account for this controversy. Our result regarding the level of IL-17 in PE women is in line with those reports indicated that the level of IL-17 in PE women was significantly increased. However, we detected increase of IL-17 levels only in the placental blood samples from PE women but not in the peripheral blood. Evaluation of the expression of IL-17A cytokine at mRNA levels indicated that fetal parts of the placenta from both patients and healthy pregnant women produce more IL-17A compared with the maternal parts. This finding indicated that the fetal part of the placenta plays a more significant role in the induction of the inflammatory responses in the placenta compared with the maternal part. Considering that all cases and controls were investigated in the term and delivered by caesarian section, the elevated expression of IL-17A by fetal part of the placenta may account as a normal inflammatory responses to prepare the placenta for delivery or because of the caesarian procedure.

In summary, the findings of the present study as the first report, indicated that the effect of IL-17 cytokine in preeclampsia is not due to the studied cytokine polymorphisms but local production of IL-17 might affect predisposition to the PE disease. Repeating the present study in other populations, investigation of other adjacent functional IL-17A SNPs and confirmation of our results using larger sample size is highly recommended.

ACKNOWLEDGEMENTS

Some parts of this study were extracted from the M.Sc. thesis written by FahimehAnvari and financially supported by Shiraz University of Medical Sciences [Grant No. 92-6581], and Student Research Committee of Shiraz University of Medical Sciences, Shiraz, Iran. The authors are grateful for Ms. A. Keivanshekouh at the Research Improvement Center of Shiraz University of Medical Sciences for improving the use of English in the manuscript.

REFERENCES

1. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet. 2001; 357:53-6.
2. Noris M, Perico N, Remuzzi G. Mechanisms of Disease: pre-eclampsia. Nat ClinPract Nephrol. 2005; 1:98-114.

3. Aali BS, Ghafoorian J, Mohamad-Alizadeh S. Severe pre-eclampsia and eclampsia in Kerman, Iran: complications and outcomes. Med Sci Monit. 2004;10:CR163-7.
4. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annu Rev Pathol. 2010; 5:173-92.
5. Petla LT, Chikkala R, Ratnakar KS, Kodati V, Sritharan V. Biomarkers for the management of pre-eclampsia in pregnant women. Indian J Med Res. 2013; 138:60-7.
6. Pennington KA, Schlitt JM, Jackson DL, Schulz LC, Schust DJ. Preeclampsia: multiple approaches for a multifactorial disease. Dis Model Mech. 2012; 5:9-18.
7. Nilsson E, Salonen Ros H, Cnattingius S, Lichtenstein P. The importance of genetic and environmental effects for preeclampsia and gestational hypertension: A family study. BJOG. 2004; 111:200-6.
8. Tanaka M, Jaamaa G, Kaiser M, Hills E, Soim A, Zhu M, et al. Racial disparity in hypertensive disorders of pregnancy in New York State: a 10-year longitudinal population-based study. Am J Public Health. 2007; 97:163-70.
9. Bulmer JN. Immune aspects of pathology of the placental bed contributing to pregnancy pathology. Baillieres Clin Obstet Gynaecol.1992; 6:461-88.
10. Peck A, Mellins ED. Plasticity of T-cell phenotype and function: the T helper type 17 example. Immunology. 2010; 129:147-53.
11. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005; 6:1123-32.
12. Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, et al. Cutting edge: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J Immunol. 2001; 167:4137-40.
13. Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, et al. Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. J Immunol. 2001; 167:4430-5.
14. Paradowska-Gorycka A, Wojtecka-Lukasik E, Trefler J, Wojciechowska B, Lacki JK, Maslinski S. Association between IL-17F gene polymorphisms and susceptibility to and severity of rheumatoid arthritis (RA). Scand J Immunol. 2010; 72:134-41.
15. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. Circulating and decidual Th17 cell levels in healthy pregnancy. Am J ReprodImmunol. 2010; 63:104-9.
16. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, et al. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009; 183:7023-30.
17. Nordang GB, Viken MK, Hollis-Moffatt JE, Merriman TR, Førre ØT, Helgetveit K, et al. Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. Rheumatology. 2009; 48:367-70.
18. Arisawa T, Tahara T, Shibata T, Nagasaka M, NakamuraM, Kamiya Y, et al. The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. J ClinImmunol. 2008; 28:44-9.
19. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-γ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. Hum Immunol. 2000; 61:863-66.
20. Chen B, Zeng Z, Hou J, Chen M, Gao X, Hu P. Association of interleukin-17F 7488 single nucleotide polymorphism and inflammatory bowel disease in the Chinese population. Scand J Gastroenterol. 2009; 44:720-6.
21. Kawaguchi M, Takahashi D, Hizawa N, Suzuki S, Matsukura S, Kokubu F, et al. IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. J Allergy Clin Immunol. 2006; 117:795-801.
22. Seiderer J, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack C, et al. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): up regulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. Inflamm Bowel Dis. 2008; 14:437-45.
23. Vanderlelie J, Gude N, Perkins AV. Antioxidant gene expression in preeclamptic placentae: a preliminary investigation. Placenta. 2008; 29:519-22.
24. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. J Clin Endocrinol Metab. 2001; 86:2505-12.

25. Saito S, Nakashima A, Shima T, Ito M. Th1 ⁄ Th2 ⁄ Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010; 63:601-10.
26. Park H, Li Z, Yang XO, Chang SH, Nurieva R, WangYH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005; 6:1133-41.
27. Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. Periodontol 2000. 2012; 58:37-68.
28. Arisawa T, Tahara T, Shibata T, NagasakaM, Nakamura M, Kamiya Y, et al .Genetic polymorphisms of molecules associated with inflammation and immune response in Japanese subjects with functional dyspepsia. Int J Mol Med. 2007; 20:717-23.
29. Arisawa T, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, et al. Functional polymorphismsin the promoter region of macrophage migration inhibitory factor and chronic gastritis. Int J Mol Med. 2007; 20:539-44.
30. Najafi S, Hadinedoushan H , Eslami G, Aflatoonian A. Association of IL-17A and IL-17 F gene polymorphisms with recurrent pregnancy loss in Iranian women. J Assist Reprod Genet. 2014; 31:1491-6.
31. Jianjun Z, Yali H, Zhiqun W, Mingming Z, Xia Z. Imbalance of T-cell transcription factors contributes to the Th1 type immunity predominant in pre-eclampsia. Am J ReprodImmunol. 2010; 63:38-45.
32. Saito S, Tsukaguchi N, Hasegawa T, Michimata T, Tsuda H, Narita N. Distribution of Th1, Th2, and Th0 and Th1/ Th2 cell ratios in human peripheral and endometrial T cells. Am J Reprod Immunol. 1999; 42:240-5.
33. Toldi G, Rigo´ J Jr, Stenczer B, Va´sa´rhelyi B, Molvarec A. Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia. Am J Reprod Immunol. 2011; 66:223-9
34. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. J Reprod Immunol. 2012; 93:75-81.
35. Ozkan ZS, Simsek M, Ilhan F, Deveci D, Godekmerdan A, Sapmaz E. Plasma IL-17, IL-35, interferon-γ, SOCS3 and TGF-β levels in pregnant women with preeclampsia, and their relation with severity of disease. J Matern Fetal Neonatal Med. 2014; 27:1513-7.