Interleukin-17 Serum Levels and TLR4 Polymorphisms in Ulcerative Colitis

Mojgan Mohammadi[1,2], Mohammad Javad Zahedi[3], Amin Reza Nikpoor[2], Mohammad Reza Baneshi[4], Mohammad Mahdi Hayatbakhsh[3*]

[1]Physiology Research Centre, [2]Department of Microbiology, Virology and Immunology, Medical School, [3]Department of Gastroenterology, Afzalipour Hospital, [4]Modeling in Health Research Center, Institute of Future Studies in Health, Kerman University of Medical Sciences, Kerman, Iran

ABSTRACT

Background: Inflammatory bowel disease, an autoimmune disease, has two clinical manifestations including Crohn's disease and ulcerative colitis (UC). IL-17 has been the target of intensive research in autoimmune diseases. The influence of Toll like receptor 4 (TLR-4) gene polymorphisms on IL-17 production has also been revealed in UC patients and tissue inflammation in mice. **Objectives:** To investigate the association between the TLR-4 gene polymorphisms, Asp299Gly and Thr399Ile and IL-17 serum levels with ulcerative colitis. Additionally, we aimed to study modulation effects of forenamed gene polymorphisms on IL-17 serum levels in UC patients and controls. **Methods:** A total of 256 healthy controls and 85 UC patients enrolled in our study. DNA was extracted and PCR-RFLP technique was employed to determine Asp299Gly and Thr399Ile polymorphisms in TLR-4 gene and IL-17 serum levels were measured by ELISA method. **Results:** There was no significant difference between the frequency of Asp299Gly A>G and Thr399Ile C>T in UC patients and controls. While IL-17 serum levels in UC patients were significantly higher than controls (p=0.003), no significant difference in IL-17 levels between different genotypes existed. Additionally, a significant inverse relationship was observed between hemoglobin level and IL-17 serum levels in UC patients (p=0.039). **Conclusions:** Increased IL-17 serum levels in our UC patients might be explained through the synergistic activity of IL-17/IL-23 axis and pro-inflammatory cytokines, causing severe clinical outcome in patients with IBD. The prolonged excretion of blood in stool driven by inflammatory process which causes iron metabolism disorder and anemia may elucidate the inverse correlation between hemoglobin and IL-17 serum levels in UC patients. Lack of association between the TLR-4 gene polymorphisms and UC in our study was consistent with the results from other Caucasian populations.

Mohammadi M, et al. Iran J Immunol. 2013; 10(2):83-92

Keywords: Asp299Gly, Gene, IL-17, Thr399Ile, Toll-Like Receptor 4, Ulcerative Colitis

---

*Corresponding author: Dr. Mohammad Mahdi Hayatbakhsh, Department of Gastroenterology, Afzalipour hospital, Kerman University of Medical Sciences, Kerman, Iran, Tel: (+) 3413222270, Fax: (+) 98 3413222270, e-mail: m24672@yahoo.com

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are two clinical presentations of inflammatory bowel disease (IBD) that affects the lining of the large intestine, causing congestion, edema and ulceration of the mucosa. IBD is a multi-factorial disease with different causes including genetic, innate immune status of the individual and environmental factors (1). Several studies have been performed in order to determine genetic factors in the development of IBD representing candidate genes for the disease such as CARD15/NOD2, TNF-α, MDR1 and IL-23 receptor (2-5). After the discovery of Toll like Receptor 4 (TLR4) gene in 1998, many studies were focused on TLR4 gene polymorphisms and their association with various diseases (6-11). From different types of mutations recorded in the TLR4 gene, two single nucleotide polymorphisms (SNPs), Asp299Gly (rs4986790) and the Thr399Ile (rs4986791) in this receptor are reported to be more important (7). However, there are controversial reports of TLR4 gene polymorphisms association with UC and CD among races (12-16). On the other hand, the cytokines are known as one of the main immunologic mediators to control physiological dysfunction in IBD (17). Additionally, loss of balance of the T cell subsets, such as T regulatory, Th1, Th2, and Th17 and consequently variation in the secretion of cytokines from these cells influences susceptibility to IBD (18). IL-17 is one of the cytokines that is considered to have important role in immunological disorders such as chronic inflammation (19) and IBD (20-24). Moreover, it appears that IL-23 plays a major role in pathogenesis of IBD (25) and activation of the IL-23/ IL-17 axis has fundamental role in this disease (24). Additionally, results from an animal model show that disease-associated tissue inflammation is correlated with IL-17 through TLR-4 signaling and an interaction between these genetics and immunological factors may introduce new insights for better understanding of pathology and treatment of chronic inflammatory disease (26). The rationale for the present study was to investigate the allele frequency of TLR4 gene at positions rs4986790, rs4986791 and IL-17 serum levels in a sample of Iranian population in order to determine the relationship between these factors and UC in our population. Another aim of our study was to look into the influence of TLR4 gene polymorphisms on IL-17 serum levels in patients with ulcerative colitis as well as healthy controls.

MATERIALS AND METHODS

Subjects. This case-control study was designed to determine the association of IL-17 serum levels and TLR4 gene polymorphisms with UC. A total of 256 healthy controls from Kerman Blood Transfusion Centre and 85 patients with UC enrolled in our study. Variables such as age, gender, experimental results related to genetic analysis of TLR-4 gene polymorphisms and IL-17 serum levels were established for all participants. Occupation, education, duration of disease, smoking, consumption of narcotic drugs, family history of disease and appendectomy, use of OCP, immunosuppressive drugs, hemoglobin level, ESR, CRP, bowel movements, blood in the stool, temperature more than 37.5 degrees, tachycardia and severity of disease were determined for UC patients, too. All patients underwent endoscopic investigation to diagnose UC according to the protocol of American Gastroenterology Association (27). Clinical history, and disease status was confirmed by a gastroenterologist. Demographic and clinical data are summarized in Table 1.

Table 1. Demographic and clinical characteristics of the study population.

Variables	UC No. (%)	Control No. (%)
Gender		
Male	38 (44.7)	136 (53,12)
Female	47 (55.3)	120 (46,87)
Age (yr)	38 ± 16	37 ± 12
Range	(84-14)	(66-19)
Disease duration (yr)	3.44 ± 3.07[@]	-
Bowel movements		
Mild	45 (52.9)	-
Moderate	28 (32.9)	-
Severe	12 (14.1)	-
Immunosuppressive drugs		
Cytotoxic and steroidal	14 (16.5)	-
ASA	40 (47.1)	-
others	31 (36.4)	-
Anemia		
Mild	41 (48.2)	-
Moderate	35 (41.2)	-
Severe	9 (10.6)	-
Blood in Stool		
Mild	39 (45.9)	-
Moderate	26 (30.6)	-
Severe	20 (23.5)	-
Tachycardia		
Mild	72 (84.7)	-
Severe	13 (15.3)	-
ESR		
Mild	58 (68.2)	-
Severe	27 (31.8)	-
Age at diagnosis (yr)	15.49 ± 34.72	-
Range	(82-11)	-
Appendectomy	(3.5) 3	-
Oral contraceptive consumption (female)	10 (21.27)	-
Smoking habit	5 (5.9)	-
Opium consumption	14 (16.5)	-
Family history of disease	8 (9.4)	-
Endoscopic criteria		
Mild	44 (51.8)	-
Moderate	25 (29.4)	-
Severe	16 (18.8)	-
Total	**85**	**256**

@ Mean ± SD

Sample Preparation. DNA was extracted from 5 ml blood samples containing EDTA and TLR4 gene polymorphisms including Asp299Gly (rs4986790) and Thr399Ile (rs4986791) were determined by PCR-RFLP (28). The sequence of the primers and the restriction enzymes are summarized in Table 2.

Table 2. Primer sequences and restriction enzymes for TLR4 genotyping.

Gene Location	Primers	PCR Product/ Enzyme	Genotyping
Asp299Gly (rs4986790)	5′-ATTAGCATACTTAGACTACTACCTCCATG-3′ 5′-GATCAACTTCTGAAAAAGCATTCCCAC-3′	249bp NcoI	**Wild type** (AA allele): 249 **Heterozygous** (AG allele): 249+223+26 **Homozugous** (GG allele): 223+26
Thr399Ile (rs4986791)	5′-GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA-3′ 5′- ACCTGAAGACTGGAGAGTGAGTTAAATGCT-3′	407bp HinfI	**Wild type** (CC allele): 407 **Heterozygous** (CT allele): 407+ 378+29 **Homozugous** (TT allele): 378+29

PCR-RFLP. PCR amplifications were performed based on the following conditions: initial denaturation at 96°C for 2 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 35 s, extension at 72°C for 30 s and final extension at 72°C for 10 min. Ten microliters of PCR products were digested by 0.5-0.25 U of allele specific restriction endonucleases NcoI (Asp299Gly) or HinfI (Thr399Ile). The restriction fragments were separated by electrophoresis on 2% agarose gels containing ethidium bromide and visualized by UV light. IL-17 serum levels were determined by ELISA technique (U- Cytech, Netherlands).

Statistical Analysis. Statistical analyses such as logistic, correlation, independent *t*-test, Chi-square, regression and descriptive statistic were performed by using SPSS software version 17.0. P values less than 0.05 were considered statistically significant.

Ethical Considerations. The study protocol was approved by the ethics committee of Kerman University of Medical Sciences and written informed consent was obtained from each patient before enrollment in the study.

IL-17 serum levels were determined by ELISA technique according to the manufacture's instruction manual (U- Cytech, Netherlands).

RESULTS

In this study, 256 healthy individuals (120 females and 136 males) with mean age of 37 ± 12 years and 85 patients with UC (38 males and 47 females) with mean age of 38 ± 16 years were included. The analysis of TLR-4 gene polymorphisms showed no significant difference in the frequencies of Asp299Gly (rs4986790) and Thr399Ile (rs4986791) between the patients and controls (Table 3). Genotype distributions were in Hardy-Weinberg equilibrium both in patients and controls.

Table 3. Genotype and allele frequencies of Asp299Gly (rs4986790) and Thr399Ile (rs4986791) of TLR-4 gene in patients with ulcerative colitis compared to the controls.

TLR4	Genotyping	UC		Control		p	Allele	UC		Control	
		N	%	N	%			N	%	N	%
Asp299Gly	AA	75	88.2	216	84.4	0.38	A	160	94.1	471	92.2
(rs4986790)	AG	10	11.8	39	15.2		G	10	5.9	40	7.8
	GG	0	0	1	0.4						
Thr399Ile	CC	74	87	212	82.8	0.36	C	159	93.5	467	91.4
(rs4986791)	CT	11	13	43	16.8		T	11	6.5	44	8.6
	TT	0	0	1	0.4						

On the other hand, significant relationship between Thr399I1e and involvement of colon was seen in endoscopic view of patients' colon. According to these results, patients carrying the polymorphism Thr399Ile showed milder degrees of disease in the endoscopic view (Table 4).

Linkage disequilibrium analysis was performed by using Chi-square test for Asp299Gly and Thr399Ile gene polymorphisms (D'= 0.803, r^2 =0.58, Pearson Chi-square=526.8) and our data showed strong linkage ($p \leq 0.001$) between these two polymorphisms which is similar to the recent published data about variability of the above mentioned TLR-4 gene polymorphisms in different ethnic groups of Iran (29).

Significant difference for the serum level of IL-17 was detected between the patient and control groups. The patient group had significantly higher serum level of IL-17 in comparison with controls (Table 5).

Interestingly, a significant inverse relationship was also observed between hemoglobin levels and IL-17 so that one unit increase in IL-17 was associated with 0.22 units decline in hemoglobin level (p=0.039, correlation= -0.022).

Table 4. Categorization of UC patients according to the severity of disease and TLR-4 genotyping of Asp299Gly A>G and Thr399Ile C>T polymorphisms.

TLR4	Genotype (N)	Severity of disease N (%)			P value	Odds Ratio
		Mild	Moderate	Severe		
Asp299Gly (rs4986790)	AA (75)	37 (49.3)	24 (32)	14 (18.7)	0.335	-
	AG (10)	7 (70)	1 (10)	2 (20)		
	GG (0)	0 (0)	0 (0)	0 (0)		
Thr399Ile (rs4986791)	CC (74)	34 (45.9)	24 (32.5)	16 (21.6)	0.019	0.120
	CT (11)	10 (90.9)	1 (9.1)	0 (0)		
	TT (0)	0 (0)	0(0)	0 (0)		

Additionally, we divided patients based on their employment status into various groups, including housewives, employees, self-employed, unemployed and students or pupils. Interestingly, we detected a significant difference in IL-17 serum levels between the groups (p=0.044). The highest level of IL-17 was found in the student or pupil group, (61.67 ± 99.42 pg/ml), and the lowest was in the unemployed subjects (3.12 ± 1.03 pg/ml). On the other hand, we did not see any influence of TLR-4 gene polymorphisms on IL-17 serum levels in UC patients and controls (Table 6).

Table 5. IL-17 serum levels in UC patients and controls.

Groups	N	IL-17(pg/ml) Mean (± SD)	P value, (95% CI)
Control	256	9.69 ± 22.77	0.003, (CI:-21.73 , -4.67)
UC	85	22.89 ± 17.57	

DISCUSSION

The results of present study showed no relationship between the Asp299Gly A>G and Thr399Ile C>T polymorphisms in the TLR-4 gene and UC in our population. Allele and genotype distributions in our study were more similar to the results of European and Caucasian populations (14-16). Lack of association between ulcerative colitis and Asp299Gly A>G and Thr399Ile C>T gene polymorphisms has been reported from the Netherlands and some of the European populations, as well (12,13,30). On the other hand, the correlation between the forenamed gene polymorphisms and ulcerative colitis

has been shown in some other populations (31-36). Difference between races and populations might explain the controversy between the published data around the world. Endoscopic view of the colon is one of the clinical indexes for diagnosing of IBD and categorizing of UC patients into, severe, mild and moderate according to their severity of disease. Our results did not show any relationship between endoscopic appearance of UC patients colon and Asp299Gly A>G gene polymorphism.

Table 6. Association of IL-17 serum levels in UC patients and controls with Asp299Gly A>G and Thr399Ile C>T genotypes of TLR-4.

TLR4	UC Genotype (N)	IL-17(pg/ml) Mean ± SD	P (CI 95%)	Control Genotype (N)	IL-17(pg/ml) Mean ± SD	P (CI 95%)
Asp299Gly	AA (75)	21.51 ± 56.83	0.545	AA (216)	9.88 ± 24.04	0.610
(rs4986790)	AG (10)	33.26 ± 61.75	(-50.16, 26.67)	AG (39)	7.85 ± 13.38	(-5.77, 9.82)
	GG (0)	0		GG (1)	41.20	
Thr399Ile	CC (74)	20.65 ± 56.76	0.352	CC (212)	8.89 ± 20.75	0.218
(rs4986791)	CT (11)	37.97 ± 60.36	(-54.08, 19.45)	CT (43)	13.54 ± 30.71	(-12.07, 2.76)
	TT (0)	0		TT (1)	41.20	

On the contrary, a significant association was detected between Thr399Ile C>T gene polymorphism and disease severity in endoscopic view in UC patients which may indicate protective role of this genotype for patients with UC. Other researchers have not reported such a correlation between the above-mentioned polymorphisms and intestinal endoscopic view of UC patients. Therefore, to our knowledge these results are the first report of its kind and valuable because of its novelty with regard to association of Thr399Ile C>T gene polymorphism and severity of UC. UC is a multi-factorial disease and immunological factors can collaborate with genetics for increasing the susceptibility to the disease. Therefore, another goal of our research was to investigate IL-17 serum levels in UC patients and comparing with healthy controls. Our results showed a significant difference in IL-17 serum level between cases and controls. We searched a number of literatures which suggest that increasing in the level of IL-17 in supernatant of cultured cells extracted from clonal biopsies or elevation of IL-17 serum or plasma levels in patients might be associated with IBD (22,37-39). By initiation of inflammation in the colon of patients, serum level of IL-17 might be elevated and an inflammation cascade is launched which causes recruitment of immune competent cells in the area. Additionally, increasing in the level of IL-17 might be explained through the IL-17/IL-23 axis which triggers synergistic activity of a group of pro-inflammatory cytokines, resulting in increased inflammation and severe clinical outcome in patients with IBD (40). On the other hand, cytokine and chemokine production are shown to be modified by TLR-4 gene polymorphisms and innate immune responses are activated by TLR-4 signaling. Results of recent studies reveal an association between TLR-4 gene polymorphism and inflammatory cytokines such as IL-17 in biopsies of colon of UC

patients (34). Additionally, multiple tissue inflammation and wasting in mice can be induced by IL-17 through TLR-4 signaling (26). However, our results showed no statistically significant influence of TLR-4 gene polymorphisms on serum level of IL-17 in UC patients and controls. Interestingly, we could find a significant inverse relationship between serum levels of interleukin-17 and hemoglobin level in patients with ulcerative colitis. Result of recent studies indicate that inflammation status in the ulcerative colitis patient increases the production of inflammatory cytokines such as IL-1, IL-6, TNF-α, TGF-β, IFN-γ and IL-17 caused erythrocytosis suppression due to direct effects of these cytokines on hematopoesis which finally results in anemia. Influence of the above mentioned cytokines on the hematopoesis is performed in many ways including: a) Interfering in delivery of iron from plasma to bone marrow which is used in hematopoesis, b) Direct inhibitory effect of IFN-γ on erythrocytosis, c) Inhibition of erythropoietin production which is affected by IL-1, IL-6 and TNF-α, d) Ability of IL-17 to induce apoptosis of hematopoietic cell precursor. Additionally, in patients with chronic diseases, including IBD, prolonged inflammatory processes results in increasing excretion of blood in stool and consequently caused iron metabolism disorder due to inadequate absorption of iron and makes patients prone to iron deficiency anemia. Decreased hemoglobin level which is associated with increased IL-17 in our UC patients might be explained by employing of this knowledge (41-43). On the other hand, we could see a meaningful relationship between IL-17 and occupation in patients with ulcerative colitis. The highest level of IL-17 was detected in students either in school or in the university. Work conditions and social stress may affect the immune system and causes elevation of IL-17. Increased levels of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α and subsequent changes in the Th1/Th2 response profiles in psychological stress status from other researches can confirm and explain our results (44,45). Taken together, our results show no relationship between the TLR4 gene polymorphisms including Asp299Gly and Thr399Ile with UC. However, mean serum levels of IL-17 serum levels in patients with UC were significantly higher than controls ($p=0.04$). Studying the profile of other cytokines may be beneficial for identifying mechanisms that exacerbate autoimmune diseases such as IBD which might help for innovation in the management and treatment of this disease.

ACKNOWLEDGEMENTS

These data have been extracted from the thesis of Amin Reza Nikpoor, M.Sc. in Clinical Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran. Special acknowledgement should be dedicated to the staff members of Kerman Blood Transfusion Centre who helped us with blood collection from healthy volunteers. This research was financially supported by Physiology Research Centre, Kerman University of Medical Sciences, under grant number 90.74.

REFERENCES

1 Yun J, Xu CT, Pan BR. Epidemiology and gene markers of ulcerative colitis in the Chinese. World J Gastroenterology. 2009; 15:788-803.

2 Cavanaugh J, IBD International Genetics Consortium. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: Crohn disease and chromosome 16. Am J Hum Genet. 2001; 68:1165-71.

3 Bonen DK, Cho JH. The genetics of inflammatory bowel disease. Gastroenterology. 2003; 124:521-36.
4 Schwab M, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, et al. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. Gastroenterology. 2003; 124:26-33.
5 Hayatbakhsh MM, Zahedi MJ, Shafiepour M, Nikpoor AR, Mohammadi M. IL-23 Receptor Gene rs7517847 and rs1004819 SNPs in Ulcerative Colitis. IranJ Immunol. 2012; 9:128-35.
6 Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science. 1998; 282:2085-8.
7 Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet.. 2000; 25:187-91.
8 Michel O, LeVan TD, Stern D, Dentener M, Thorn J, Gnat D, et al. Systemic responsiveness to lipopolysaccharide and polymorphisms in the toll-like receptor 4 gene in human beings. J Allergy clin Immun. 2003; 112:923-9.
9 Allen A, Obaro S, Bojang K, Awomoyi AA, Greenwood BM, Whittle H, et al. Variation in Toll-like receptor 4 and susceptibility to group A meningococcal meningitis in Gambian children. Pediatr Infect Disease J. 2003; 22:1018-9.
10 Read RC, Pullin J, Gregory S, Borrow R, Kaczmarski EB, di Giovine FS, et al. A functional polymorphism of toll-like receptor 4 is not associated with likelihood or severity of meningococcal disease. J Infect Dis. 2001; 184:640-2.
11 Faber J, Meyer CU, Gemmer C, Russo A ,Finn A, Murdoch C, et al. Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy. Pediatr Infect Dis J. 2006; 25:80-1.
12 Oostenbrug LE, Drenth JP, de Jong DJ, Nolte IM ,Oosterom E, van Dullemen HM, et al. Association between toll-like receptor 4 and inflammatory bowel disease. Inflamm Bowel Dis. 2005; 11:567-75.
13 Rigoli L, Romano C, Caruso RA, Presti MA, Di Bella C, Procopio V, et al. Clinical significance of NOD2/CARD15 and Toll-like receptor 4 gene single nucleotide polymorphisms in inflammatory bowel disease. World J Gastroenterolog. 2008; 14:4454-61.
14 Guo Q, Xia B, Jiang Y, Morré S, Cheng L, Li J, et al. Polymorphisms of CD14 gene and TLR4 gene are not associated with ulcerative colitis in Chinese patients. Postgrad Med J. 2005; 81:526-9.
15 Hume GE, Fowler EV, Doecke J, Simms LA, Huang N, Palmieri O, et al. Novel NOD2 haplotype strengthens the association between TLR4 Asp299gly and Crohn's disease in an Australian population. Inflamm Bowel Dis. 2008; 14:585-90.
16 Shen X, Shi R, Wang Y, Zhang H, Zhou X, Shen F, et al. Toll-like receptor gene polymorphisms and susceptibility to inflammatory bowel disease in Chinese Han and Caucasian populations. Zhonghua Yi Xue Za Zhi. 2010; 90:1416-20.
17 Jump RL, Levine AD. Mechanisms of natural tolerance in the intestine. Implications for inflammatory bowel disease. Inflamm Bowel Dis. 2004; 10:462-78.
18 Leon F, Smythies LE, Smith PD, Kelsall BL. Involvement of dendritic cells in the pathogenesis of inflammatory bowel disease. Adv Exp Med Biol . 2006; 579:117-32.
19 Yamada H. Current perspectives on the role of IL-17 in autoimmune disease. J Inflamm Res. 2010; 3:33-44.
20 Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, et al. Regulation of inflammatory responses by IL-17F. J Exp Med. 2008; 205:1063-75.
21 O'Connor Jr W, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, et al. A protective function for interleukin 17A in T cell–mediated intestinal inflammation. Nat Immunol. 2009; 10:603-9.
22 Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut. 2003; 52:65-70.
23 Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, et al. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: Elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. Inflamm Bowel Dis. 2005;11:16-23.
24 Hölttä V, Klemetti P, Sipponen T, Westerholm-Ormio M, Kociubinski G, Salo H, et al. IL-23/IL-17 immunity as a hallmark of Crohn's disease. Inflamm Bowel Dis. 2008; 14:1175-84.
25 Mohammadi M, Hayatbakhsh MM, Zahedi MJ, Jalalpour MR, Pakgohar A. Serum Interleukin-23 Levels in Patients with Ulcerative Colitis. Iran J Immunol. 2011;8:183-8.
26 Tang H, Pang S, Wang M, Xiao X, Rong Y, Wang H, et al. TLR4 activation is required for IL-17-induced multiple tissue inflammation and wasting in mice. J Immunol. 2010; 15:185:2563-9.
27 Kornbluth A, Sachar DB . Ulcerative colitis practice guidelines in adults: American college of gastroenterology, practice parameters committee. Am J Gastroenterol. 2010;105:501-23.
28 Folwaczny M, Glas J, TÖRÖK HP, Limbersky O, Folwaczny C. Toll-like receptor (TLR) 2 and 4 mutations in periodontal disease. Clin Exp Immunol. 2004;135:330-5.
29 Ioana M, Ferwerda B, Farjadian S, Ioana L, Ghaderi A, Oosting M, et al. High variability of TLR4 gene in different ethnic groups in Iran. Innate Immun. 2012; 18:492-502.
30 Tahara T, Arisawa T, Shibata T, Hirata I, Nakano H. Absence of common polymorphisms of Toll like receptor 4 (TLR4 :(Asp299Gly, Thr399Ile in patients with gastroduodenal diseases in Japan. J Clin Biochem Nutr. 2007; 40:62.
31 Braat H, Stokkers P, Hommes T, Cohn D, Vogels E, Pronk I, et al. Consequence of functional Nod2 and Tlr4 mutations on gene transcription in Crohn's disease patients. J Mol Med. 2005; 83:601-9.
32 Török HP, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. Clin Immunol. 2004; 112:85-91.
33 Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, et al. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut. 2004;53:987-92.
34 Pierik M, Vermeire S, El-Housni H, Claessens G, Quertinmont E, Joosens S, et al. Toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with ulcerative colitis (UC). Gastroenterology. 2003;124:A370.
35 Meena NK, Verma R, Verma N, Ahuja V, Paul J. TLR4 D299G Polymorphism Modulates Cytokine Expression in Ulcerative Colitis. J Clinl Gastroenterol. 2013; [Epub ahead of print].
36 Shen X, Shi R, Zhang H, Li K, Zhao Y, Zhang R. The Toll-like receptor 4 D299G and T399I polymorphisms are associated with Crohn's disease and ulcerative colitis: a meta-analysis. Digestion. 2010; 81:69-77.
37 Hundorfean G, Neurath MF, Mudter J. Functional relevance of T helper 17 (Th17) cells and the IL-17 cytokine family in inflammatory bowel disease. Inflamm Bowel Dis. 2012; 18:180-6.

38 Xiong-jun L, Ze-lin M, Ya-jing Z. The clinical significance of interleukin 4 and interleukin 17's blood plasma level in patients with ulcerative colitis. Youjiang Medical Journal. 2005.
39 Rovedatti L, Kudo T, Biancheri P, Sarra M, Knowles C, Rampton DS, et al. Differential regulation of interleukin 17 and interferon γ production in inflammatory bowel disease. Gut. 2009; 58:1629-36.
40 Bogaert S, Laukens D, Peeters H, Melis L, Olievier K, Boon N, et al. Differential mucosal expression of Th17-related genes between the inflamed colon and ileum of patients with inflammatory bowel disease. BMC immunology. 2010;11:11-61.
41 Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med. 2009; 361:888-98.
42 Broxmeyer HE, Starnes T, Ramsey H, Cooper S, Dahl R, Williamson E, et al. The IL-17 cytokine family members are inhibitors of human hematopoietic progenitor proliferation. Blood. 2006;108:770.
43 Gu Y, Hu X, Liu C, Qv X, Xu C. Interleukin (IL)-17 promotes macrophages to produce IL-8, IL-6 and tumour necrosis factor-α in aplastic anaemia. Br J Haematol. 2008; 142:109-14.
44 Starnes T, Broxmeyer HE, Robertson MJ, Hromas R .Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. J Immunol. 2002;169:642-6.
45 Steptoe A, Willemsen G, Owen N, Flower L, Mohamed-Ali V. Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. Clin Sci. 2001;101:185-92.