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Analysis of Cytokine Gene Polymorphism Allelic Variation in the Turkish Population with Inflammatory Bowel Disease.

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Abstract

Inflammatory bowel diseases (IBD), Crohn's Disease (CD) and Ulcerative Colitis (UC) are complex diseases showing genetic heterogeneity that arise with the interaction of the genetic and environmental agents. The prevalence of the cytokine gene polymorphism has different distributions in the various races of the world. In our study, 22 polymorphic regions of 13 different cytokine genes were evaluated in IBD patients in the Black Sea Region of Turkey. Sixty-nine IBD patients (18 with Crohn's disease and 51 with ulcerative colitis) and 100 healthy individuals as controls who lived in the Black Sea Region and whose clinical diagnoses were realized in Research Hospitals, were selected. DNA was isolated from the blood of the selected individuals and the analyses of the cytokine gene polymorphisms were determined with the method of PCR-SSP (sequence specific primer). When the patients were compared with the control group in terms of cytokine genes, significant changes were observed in the allelic frequencies of the diseased group compared to the healthy individuals thus implying a role of allelic frequencies in Crohn's disease and ulcerative colitis. We conclude that, the allelic changes arising at the genomic level in the cytokine gene polymorphisms in the Turkish population could make contributions to the clinic diagnosis of patients with IBD.

Keywords: Inflammatory bowel disease , cytokine gene polymorphism, PCR-SSP, Black Sea Region, Turkish population

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Introduction

Inflammatory bowel diseases (IBD) are in the group of chronic inflammatory diseases having the indications such as abdominal cramp, rectal bleeding and diarrhea [1]. Crohn's disease (CD) and ulcerative colitis (UC) are two main forms of the inflammatory bowel disease having multi-factorial inheritance characteristics arising with the interaction of genetic and environmental factors [2]. There is a continuing trend towards an increasing incidence and prevalence of IBD across Asia (particularly in East Asia). While this is occurring among developing nations, it is also being seen in Japan, a socio-economically advanced country [3]. It attracts attention that the incidence and prevalence of IBD have shown significant increase in recent years in Turkey [4]. The distribution of both diseases is different in terms of age, geography, race, ethnic, and social segments [5]. In clinical cases, environmental factors, infecting agents, increase of permeability in the small bowel, autoimmune responses, and auto antibodies were also involved in these illnesses in addition to the genetic factors [6]**.** It is thought that the cytokines, which mediate inflammation in IBDs have many functions in the immune system. Also, it was determined by the researchers that the cytokines could be important in the pathogenesis of IBD [7,8,9]**.** Studies in this field have shown that with the negative changes of the environmental conditions cytokine gene fluctuations increase and this triggers IBD [10]. In IBD patients, the relationship between the candidate gene polymorphism and sensitiveness to this illness was proposed to differ among different population. In this study, we investigated the cytokine gene polymorphisms in the Black Sea Regional population and to determine the cytokine gene polymorphism changes in IBD at the allelic level.

Materials & Methods

Patients and genomic DNA extraction

This research was conducted among the Black Sea Region population, which geographically corresponds to Northern Turkey. Sixty-nine cases for this study were selected from the patients applying to the Trabzon Farabi Hospital Gastroenterology and Kocaeli Medicine Faculty Gastroenterology polyclinics with inflammatory bowel diagnosis and 100 healthy individuals as control group**.** IBD was determined using the physical examination, radiological, endoscopic, and pathological test criteria according to the complaints of the patients. From each of the 69 patient and 100 healthy individuals included in the study, 8 ml blood was taken into EDTA tubes. From the peripheral blood taken, DNA isolation was conducted with the method of Salting – Out protocol [11]. The optical density of the concentration of DNA obtained in this stage was read in the Nanodrop spectrophotometer (Thermo) in 260 nm wave length [12]**.** The isolated DNA samples were kept at -80^{o}C. The genomic analyses were completed in School of Medicine**,** Hematology Department tissue typing Laboratory.

PCR-SSP and electrophoresis

The Protrans cytokine Cycler Plate 200096; the Cytokine gene panel, which was obtained from the Protrans firm for the determination of the cytokine gene polymorphism was used to determine 22 polymorphic varieties of 13 different cytokines. It is composed of 3 different sub-components; a) plates of 96 saturated with the oligonucleotide primers, b) R buffer and c) Y buffer. For the PCR-SSP amplification of each individual, the master-mix and DNA mixture having total volume of 521,3 µl [Buffer R 138 µl, Buffer Y 280 µl, Genomic DNA (50-300 ng/µl) or 100 µl and Taq Polimeraz (5U/µl): 3,3 µl] was prepared [13]**.**

The prepared master-mix was slightly mixed in the vortex and was distributed to 44 wells in the plate with the final volume being 10 µl for each patient. For the following ones given for the cytokine genes, the beginning denaturation was amplified at 94^{o}C for 2 min, denaturation was amplified at 94^{o}C for 10 sec, annealing-extension was amplified at 65^{o}C for 1 min 10 cycles, the final denaturation was amplified at 94^{o}C sec, annealing was amplified at 61^{o}C for 50 sec, the extension was amplified at 72^{o}C for 30 sec, 30 cycles and hold was amplified at 4^{o}C in indefinite PCR cycle programmed. For the implementation of the amplified PCR products in the electrophoresis, 1XTBE buffered %2 agarose gel was prepared. The samples were executed in 1X TBE buffered environment at 75V, 50 A for 35 minutes by being loaded to 44 well gel for each patient (Perrey et al.2002). The results were executed in 1X TBE buffered environment at 75 V , 50 A for 35 minutes by being loaded to 44well gel for each patient. The result were examined in the trans illuminator at UV and the wells having double bands from the samples available in each well were accepted as positive and photographed. The changes in the cytokine gene polymorphisms of the patients were determined as a result of the comparison with the control groups within the information in the chart in the protrans cytokine 2 cycler plate system analysis table [13].

Statistical analysis

The statistical analysis of the cytokine gene polymorphisms was conducted by using the SPSS Windows 16.0.0 (Statistical package for social science). The statistical significance between the groups was calculated by using Pearson's adjusted Chi- square and Fisher's exact Chi- square tests. The significance level was selected as p=0.05 in the statistical comparisons throughout the manuscript.

Results

In total, we studied 44 sites of 22 different polymorphic regions of 13 cytokine genes in IBD. The 13 genes are interleukin-1alpha (IL-1α), interleukin-1beta (IL-1β), interleukin-1reseptor (IL-1R), interleukin-1 anti-reseptor (IL-1RA),interleukin-4 anti receptor (IL-4RA), interleukin-2(IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-12 (IL-12), interferon gamma (IFN-γ), tumor growth factor beta (TGF-β),and tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) . Of the 44 sites examined, a total of 23 (53%) sites in the IBD manifested changes in allele frequencies compared to the control group.

Among those changes, only four (10%) sites had an elevated allele frequency and the other 19 (43%) sites had a decreasing allele frequency compare to the control group allele frequencies (Table 1).

The total number of sites that had changes in allele frequency of the cytokine gene was 19 (about 44%) in terms of Crohn's disease patients in which 15 (35%) sites showed a decrease and four (10%) sites manifested increase (Table 2). We also observed that, for the ulcerative colitis the total change in cytokine gene polymorphisms was about 20 (45%) sites in which 11 (25%) sites showed decrease in allele frequency compare to the controls and 9 (20%) showed increase (Table 3). Total changes and alleles polymorphic positions were expressed in three groups (IBD, CD and UC) and control group (Table 4).

Table 1. *Statistical Values of Cytokine Gene Polymorphisms in Controls group and Inflammatory Bowel Diseases (IBD) patients*

Cytokine Genes	Position	Allel	Bp	Control	IBD	Chi-square test	Fischer test	P Value	Result
IL-1α	-899	T	220	68	17	29.2	-	0.00	Decrease
		C	220	32	38	0.199	-	0.656	-
IL-1β	-511	C	220	67	52	2.38	-	0.123	-
		T	220	33	38	0.199	-	0.656	-
IL-1β	.+3962	T	340	70	38	10.861	-	0.001	Decrease
		C	340	30	48	3.123	-	0.077	-
IL-1R	pst11970	C	290	65	37	0.851	-	0.345	-
		T	290	35	42	0.225	-	0.635	-
IL-1RA	mspa111100	T	300	21	46	7.715	-	0.005	Increase
		C	300	79	32	25.002	-	0.000	Decrease
IL-4RA	.+1902	G	140	80	48	11.335	-	0.001	Decrease
		A	140	20	43	6.148	-	0.013	Increase
IL-12	-1188	C	800	72	28	21.305	-	0.000	Decrease
		A	800	28	35	0.341	-	0.559	-
IFN- γ	UTR5644	A	280	77	10	53.467	-	0.000	Decrease
		T	280	23	14	1.165	-	0.280	-
TGF-β	codon 10	G	80	42	32	0.885	-	0.347	-
		C	80	58	73	6.202	-	0.013	-
TGF-β	codon 10	C	80	40	22	2.012	-	0.156	Decrease
		T	80	60	16	23.007	-	0.000	Decrease
TNF-α	-238	G	110	13	45	11.375	-	0.001	Decrease
		A	110	87	33	30.458	-	0.000	Decrease
TNF- α	-308	G	110	9	23	3.759	-	0.053	Increase
		A	110	91	7	81.611	-	0.000	Decrease
IL-2	-330	T	570	33	23	0.741	-	0.389	-
		G	570	67	20	25.547	-	0.000	Decrease
IL-2	.+166	G	570	75	12	45.397	-	0.000	-
		T	570	25	12	3.208	-	0.073	Decrease
IL-4	-1098	T	560	10	19	1.144	-	0.285	İncrease
		C	560	90	48	21.004	-	0.000	-
IL-4	-1098	T	560	87	32	32.105	-	0.000	-
		C	560	13	32	1.036	-	0.000	Decrease
IL-4	-590	C	610	84	14	54.141	-	0.000	Decrease
		T	610	16	6	2.298	-	0.130	-
IL-6	-174	G	430	38	32	0.248	-	0.619	Decrease
		C	430	62	10	33.520	-	0.000	Decrease
IL-6	nt565	G	430	81	14	48.412	-	0.000	-
		C	430	19	20	0.000	-	1.000	-
IL-10	-1082	G	300	48	33	2.032	-	0.135	-
		G	300	52	39	1.458	-	0.227	Decrease
IL-10	-819	C	300	48	23	6.925	-	0.009	Decrease
		A	300	52	19	13.003	-	0.000	-
IL-10	-592	C	300	75	28	23.292	-	0.000	-
		A	300	25	0	17.757	-	0.000	-

Fisher Exacts test. Value <0.05 is significant.

Table 2*. Statistical Values of Cytokine Gene Polymorphisms in Control Group and Crohn's Diseases (CD) patients.*

Cytokine Genes	Position	Allel	bp	Control	Crohn's	Chi-square test	Fischer test	P Value	Result
IL-1 α	-899	T	220	68	50	1.200	-	0.000	-
		C	220	32	56	0.081	-	0.139	-
IL-1 β	-511	C	220	67	64	0.000	-	1.000	-
		T	220	33	50	0.152	-	0.254	-
IL-1β	.+3962	T	340	70	39	4.187	-	0.041	Decrease
		C	340	30	67	5.980	-	0.014	-
IL-1R	pst11970	C	290	65	44	1.360	-	0.243	-
		T	290	35	33	3.881	-	0.049	-
IL-1RA	mspa111100	T	300	21	56	6.438	-	0.011	Increase
		C	300	79	44	6.438	-	0.011	Decrease
IL-4RA	.+1902	G	140	80	39	8.642	-	0.003	Decrease
		A	140	20	50	4.520	-	0.034	Increase
IL-12	-1188	C	800	72	44	3.277	-	0.070	-
		A	800	28	50	1.963	-	0.161	-
IFN- γ	UTR5644	A	280	77	11	22.020	-	0.000	Decrease
		T	280	23	22	-	*	1.000	-
TGF- β	codon 10	G	80	42	28	0.611	-	0.434	-
		C	80	58	39	1.249	-	0.264	-
TGF- β	codon 10	C	80	40	6	5.831	-	0.016	Decrease
		T	80	60	44	0.745	-	0.388	Decrease
TNF- α	-238	G	110	13	17	-	*	0.717	-
		A	110	87	28	18.897	-	0.000	Decrease
TNF- α	-308	G	110	9	44	-	*	0.002	İncrease
		A	110	91	11	37.907	-	0.000	Decrease
IL-2	-330	T	570	33	22	0.230	-	0.632	-
		G	570	67	17	11.025	-	0.001	Decrease
IL-2	.+166	G	570	75	17	15.673	-	0.000	-
		T	570	25	17	-	*	0.529	Decrease
IL-4	-1098	T	560	10	17	-	*	1.000	Decrease
		C	560	90	67	-	*	0.055	-
IL-4	-1098	T	560	87	44	-	*	0.001	-
		C	560	13	50	-	*	0.004	İncrease
IL-4	-590	C	610	84	22	20.344	-	0.000	Decrease
		T	610	16	11	-	*	1.000	Decrease
IL-6	-174	G	430	38	22	0.851	-	0.356	Decrease
		C	430	62	11	11.760	-	0.000	-
IL-6	nt565	G	430	81	22	16.900	-	0.000	-
		C	430	19	28	-	*	0.519	-
IL-10	-1082	G	300	48	39	0.152	-	0.697	-
		G	300	52	33	1.178	-	0.278	-
IL-10	-819	C	300	48	28	1.463	-	0.226	-
		A	300	52	28	2.230	-	0.135	Decrease
IL-10	-592	C	300	75	39	5.690	-	0.017	-
		A	300	25	0	-	*	0.015	-

Fisher Exacts test. Value <0.05 is significant.

Table 3*. Statistical Values of Cytokine Genes Polymorphisms in Control Group and Ulcerative colitis (UC) patients.*

Cytokine Genes	Position	Allel	bp	Control	UC	Chi-square test	Fischer test	P Value	Result
IL-1α	-899	T	220	68	8	15.10	-	0.000	Decrease
		C	220	32	54	-	*	0.198	-
IL-1β	-511	C	220	67	100	-	*	0.027	Increase
		T	220	33	77	-	*	0.009	Increase
IL-1β	.+3962	T	340	70	54	6.837	-	0.329	-
		C	340	30	69	-	*	0.022	Increase
IL-1R	pst11970	C	290	65	85	-	*	0.196	-
		T	290	35	85	-	*	0.196	-
IL-1RA	mspa111100	T	300	21	92	-	*	0.000	Increase
		C	300	79	92	-	*	0.433	-
IL-4RA	.+1902	G	140	80	69	-	*	0.461	-
		A	140	20	69	-	*	0.001	Increase
IL-12	-1188	C	800	72	85	-	*	0.486	-
		A	800	28	69	-	*	0.009	Increase
IFN- γ	UTR5644	A	280	77	23	-	*	0.000	Decrease
		T	280	23	8	-	*	0.270	-
TGF-β	codon 10	G	80	42	38	-	*	1.000	-
		C	80	58	54	-	*	1.000	-
TGF-β	codon 10	C	80	40	31	-	*	0.750	Decrease
		T	80	60	15	6.529	-	0.011	Decrease
TNF-α	-238	G	110	13	92	-	*	0.000	Increase
		A	110	87	54	-	*	0.019	Decrease
TNF-α	-308	G	110	9	15	-	*	0.596	Increase
		A	110	91	15	-	*	0.000	Decrease
IL-2	-330	T	570	33	46	-	*	0.350	-
		G	570	67	54	-	*	0.522	-
IL-2	.+166	G	570	75	38	-	*	0.023	Decrease
		T	570	25	23	-	*	1.000	-
IL-4	-1098	T	560	10	31	-	*	0.078	İncrease
		C	560	90	62	-	*	0.025	-
IL-4	-1098	T	560	87	54	-	*	0.019	Decrease
		C	560	13	38	-	*	0.105	Decrease
IL-4	-590	C	610	84	31	-	*	0.000	Decrease
		T	610	16	15	-	*	1.000	-
IL-6	-174	G	430	38	62	1.472	-	0.225	-
		C	430	62	46	0.515	-	0.473	-
IL-6	nt565	G	430	81	15	-	*	0.000	-
		C	430	19	31	-	*	0.461	-
IL-10	-1082	G	300	48	54	0.004	-	0.949	-
		G	300	52	62	0.091	-	0.762	-
IL-10	-819	C	300	48	54	0.004	-	0.763	-
		A	300	52	46	0.004	-	0.949	Decrease
IL-10	-592	C	300	75	38	-	*	0.023	
		A	300	25	15	-	*	0.716	-

Fisher Exacts test. Value <0.05 is significant.

***Table 4**. The polymorphic positions of the alleles in the patients (IBD, CD, and groups along with the changing and non changing alleles of polymorphic positions.*

	Total 53%);(23)	**Increasing alleles** 10%); (4)	**Decreasing alleles** **(**43%) ;(19)		**Non changing alleles** **(**47%) ;(21)	
Inflammatory Bowel Disease		IL1RA mspa111100T IL4 RA +1902A TNFα -308G IL4 -1098T	IL1 α –889T ILβ +3962T IL1RA mspa111100C IL4RA +1902G IL12 –1188C IFNγ UTR 5644A TGFβ codon 10C TGFβ codon 10T TNFα -238A/G	IL 2 -330G IL 2 +166G IL 4 -1098T IL 4 -590 C IL 4 -590 C IL 6 -174 C IL 10 -819 C TNF α 308A TNF α 308A IL10 -1082 G	IL1β-511C IL1β-511T IL1R pst11970C IL1R pst11970C TGF β codon 10G TGF β codon 10C IL6 nt565G IL6 nt565C IL10-592C IL10-592A	IL10-819A IL4-1088T IL4-590T IL6-174C IL2-166G IL4-1088C IL2-330T FN γ UTR 5644T IL12-1188A IL 1 α –889C IL β +3962
	Total 45%);(19)	**Increasing alleles** 10%); (4)	**Decreasing alleles** 35%); (15)		**Non changing alleles** **(** 55%) ; (25)	
Crohn's Diseases		IL1RA mspa111100T IL 4RA +1902A TNF α 308G IL4 -1098T	IL1β +3962T IL1RA mspa111100C IL4RA +1902G IFN γ UTR5644A TGFβ codon 10T TGF β codon 10C TNF α 238A	TNF α 308A IL2-330G IL2 +166G IL4 -1098T IL4 -590T IL4 -590C IL6 –174G IL10 -819 A	IL1β –511T IL1β –511C ILα -889T IL1 α -889C IL β +3962C IL1R pst11970C IL1R pst11970T IL12-1188A IL12-1188C IFN γ UTR 644T TGF β codon 10C TGF β codon10G	IL2 +166G IL2 +166C IL6-174C IL6-174T IL2 -330T IL4 –1098T IL4 -1098C IL10 -819 G IL10 -819 C IL6 nt565G IL6 nt565C IL10-592C IL10-592A
	Total 45%);(20)	**Increasing alleles** 20%); (9)	**Decreasing alleles** 25%); (11)		**Non changing alleles** **(**55%) ; (24)	
Ulcerative colitis		IL1β –511T ILβ +3962C 1β –511C IL 4 RA+1902A IL1RA mspa111100T IL 4 -1098T TNF α -238G TNF α -308A IL 12 -1188A	IL 1 α -889T IFN γ UTR 5644A TGF β codon 10C TGF β codon 10T TNF α -238A TNF α -308T IL 2 +166T IL 4 –1098T IL 4 -1098G IL 4 -590T IL 10 -819 C		IL2-330T Il2-330G IL1β –511C IL 1 α -889T IL1R pst11970C IL1R pst11970T IL1RA mpsa1111000C IL4RA+1902G IL12-1188C IFN γ UTR 5644T TGF β codon 10C TGF β codon 10G	IL4-1098C IL2+166T IL+-590T IL6-174G IL10 -1082G IL10 -1082C IL10 -819 C IL10-592C IL10-592A IL6 nt565G IL6 nt565C IL 6 –174C

Discussion

In this study we found that there are certain sites in the cytokine genes that had variegated polymorphic allele frequencies in IBD diseases. We conducted in depth analyses to find out the nature of the changes in the allele frequency of the each site and whether those changes could be used in clinical application of the diagnosis of the disease. Since the allele frequencies are results of accumulation of the mutants in the population, the fre-

quency of alleles can vary in different populations [14,15,16]. Among the polymorphic sites studied, TNF α -308G site was shown to increase the inflammation and IL -1RA site represses the inflammation. A similar study conducted in the Japan population indicated that the allelic differences of TNF-α and IL- 1 RA genes associated with the inflammation and TNF-α was an important candidate gene in the inflammatory bowel diseases [17]. When the results of our study are compared with the Japanese population, there is a similarity in the allele frequency patterns of TNF-α and IL-1RA genes. Nevertheless, polymorphism pattern of IL-4RA gene obtained in our study shows differences when compared to a number of other studies [18]

To investigate the possible genes associated with Crohn's disease, wide genome screenings were conducted recently and the effects of the allelic frequency changes in the polymorphic regions were discussed [19,20]. In our study, we have observed that the changes in the allele frequencies in the cytokine gene polymorphisms were less significant in CD and more significant in UC. An existence of a balance between the IL-1α and IL-1β cytokine pro-inflammators were thus proven. With an increase in the rate of IL-1 (IL-1α and IL-1β), there is inflammation in the mucosa of the patient's bowel. However, this is not the case in the healthy individuals [21]. Among the US and European population, the irregularity of pro-inflammatory cytokines (IL-1α, TNF-α, IL-12, IL-8 and IL-6) and anti–inflammatory cytokines (IL-4, IL-10, IL-1RA and IFN-γ) in IBD were reported with an increase in allele frequency [22]. Nonetheless, we conversely observed a decreasing pattern in IBD. We propose that this difference was due to the nature of the population studied. In IBD, the increases in the allele frequency of the TNF-α, IL-1, IL-2, IL-6 and IL-8 was with respect to the expression in bowel mucosa [23]**.** The elevation of allele frequency of the certain sites of TNF-α and IL-1, IL-2, IL-6 and IL-4 significantly increased in the bowel mucosa in UC and CD in the Chinese population [24,25]. In our research, in the cytokine gene polymorphisms of UC patient, there were increases in IL-1 β –511C, IL-1 β –511T, IL-1 β +3962C, and decreases in IL -1 α -889C, IL-1 β +3962C, IL -1RA mspa 111100T, and no changes in the others. In UC, the repetitions of the IL-1 RA gene polymorphisms have significantly increased. This increase was observed in Ashkenazi Jews in the state of Los Angeles [19]. In IBD, it was shown that there were decreases in the allele frequencies of the same gene and allelic frequencies of IL-1RA were shown to increase between UC and CD patients [26]. The same pattern was observed in the patients with IBD [27]. Some studies showed that some cytokines (IL-1 and IL-1RA) play roles in the formation of the disease and in the development of the inflammation in IBD [27]. Our findings here are in parallel with the studies conducted in various populations in terms of IL-1RA and TNF-α. It is reported that frequency of some alleles in the IL-1RA has significantly increased in the patients with CD and UC and the elevation leaded the increase in the formations of the lesions [28].

The effects of polymorphisms in IL-1β and IL-1 RA genes in the Europe population is associated with UC, CD, and healthy control groups and the results revealed that there is an association for one single positive allele of IL-1β gene for CD and two positive alleles for IL-1RA [29]**.** Scientists showed that there was an important decrease in the rate of IL-1/IL1-RA in the IL-1 and IL-1RA measurements that they have realized in UC, CD and control groups [30]. However, we our results differ with previous studies in terms of allele frequencies of the IL-4RA. We found that the allelic frequencies of IL-4RA as between 60-69% in UC and as 39-50% in the CD patients in the regional Turkish Community ([4]. In the recent research, the hypothesis that the IBD can be treated via cytokines and for this reason, the activity, excretion and synthesis of the cytokines and cytokine signal pathways in the target cells in the bowel mucosa immune system can be inhibited [31,32]. In our research, however, a decreasing, not an increasing pattern in the IL-2, IL-4, IL-6 polymorphisms in IBD, CD, and UC was observed.

In conclusion, we observed the cytokine genes that vary in the direction of increasing frequencies of allelic genes (eg), while IL -1RA , IL - 4RA , TNF - α and IL - 4 is similar in all the three disease groups (IBD , CD and UC) . However, UC allelic frequency of IL- 1β exhibit significant increase that maybe diverge from CD and IBD phenotypically. Pro-inflammatory cytokine, IL- 1β gene may have induced the inflammation in the UC. Moreover, this result may then be potentially used at the UC clinic to differentiate patients with UC from IBD and CD. In general, pro-inflammatory cytokines (e.g. TNF - α and IL - 4) and anti -inflammatory cytokines (IL -1RA , IL - 4RA) regulate themselves during an inflammatory reaction either by increasing or decreasing its response, respectively. Analyses of the cytokine genes in this study showed that the change in the allelic frequencies in the lower direction of the IL - 1α and TGF - β , TNF - α , IL - 2 , IL - 4 , IL - 10 and IFN - ɣ was relatively high in all disease groups (IBD , CD and UC). High rate of reduction in the allelic frequencies of cytokine genes in these groups showes that anti-inflammatory cytokines and inflammatory diseases play an important role in the diagnosis . Overall, to the best of our knowledge, We think that the changes in the direction of increasing and decreasing in the cytokine gene polymorphisms can be a factor contributing to the formation of IBD. The changes could be used in the clinical diagnosis of the patients. In addition to the formation of IBD, determination of cytokine gene polymorphism in IBD could ultimately help for clinical diagnosis of cancer, autoimmune disease, and other multifactorial genetic disorders resulting from in-

flammation. We have a positive approach to the idea that in addition to the effects of the candidate genes in the formation of the above mentioned diseases, different environmental conditions, genotypic differences in the regional populations, nutritional habits, and geographical factors could play a role.

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