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T-Helper 1, T-Helper 2, and T-Regulatory Cytokines Gene Polymorphisms in Irritable Bowel Syndrome

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Abstract—Inflammation and mucosal immune system activation have an important role in irritable bowel syndrome (IBS), whereas genetic factors can control some immunological mediators. In this study, a number of polymorphic genes coding for T-helper 1, T-helper 2, and T-regulatory cytokines were genotyped in 71 patients with IBS, and the results were compared with controls. IL-4 CC genotype at position −590, IL-4 TT genotype at position −33, and IL-10 GA genotype at position −1082 were significantly overrepresented in the patients with IBS in comparison with controls ($P<0.001$). The frequencies of the following haplotypes in the patient group were significantly higher than in the control group: IL-2 (−330, +160) GT haplotype ($P=0.002$), IL-4 (−1098, −590, −33) TCC haplotype ($P<0.001$), and TCT haplotype ($P<0.001$). While production of cytokines could be affected by genetic polymorphisms within coding and promoter regions of cytokine genes, IL-4 and IL-10 gene polymorphisms could affect individual susceptibility to IBS.

KEY WORDS: cytokine; genetic polymorphism; IL-4; IL-10; irritable bowel syndrome.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by recurrent abdominal pain and altered bowel habits [1, 2]. Several pathophysiological mechanisms have been proposed in IBS, including visceral hypersensitivity [3, 4], altered gut motility [5, 6], inflammation and mucosal immune system activation [7], and psychological factors [8–10]. Genetic factors could have also a major role in this regard [11, 12]. However, the exact etiology of IBS is still unclear,

Cytokines are important modulators in the immune responses and inflammatory reaction, which can play an important role in intestinal inflammation [13]. Production of cytokines could be affected by genetic polymorphisms within coding and promoter regions of cytokine genes [14, 15]. Therefore, genetic predisposition to produce high or low amounts of a particular cytokine may affect disease susceptibility and clinical outcome [11, 16]. The role of cytokine gene polymorphisms in individual susceptibility to certain diseases has been documented [17–22], while our understanding in IBS is limited due to few performed studies in this area.

In order to analyze the allele and genotype and haplotype frequencies of a number of polymorphic genes coding for cytokines, this study was performed in a group of patients with IBS and compared with healthy control subjects.

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PATIENTS AND METHODS

Subjects

This project was approved by the Ethical Committee of Tehran University of Medical Sciences. Written informed consent was obtained from all subjects before sampling. Seventy one unrelated patients with IBS (22 male, 49 female), with mean age of 41.7±17.0, who were referred to our center, Tehran, Iran, during the year 2008, were investigated in this study. IBS, defined according to the Rome III criteria as recurrent abdominal pain or discomfort for at least 3 days per month during the previous 3 months, is associated with two or more of the following symptoms: improvement with defecation, onset associated with a change in the frequency of stools, and/or onset associated with a change in form or appearance of stools [23, 24].

One hundred and forty healthy unrelated control subjects were also randomly selected from blood donors at Iranian blood transfusion organizations in Tehran [25].

Genotyping

After DNA extraction from whole blood, using salting out method, cytokine genes typing was performed by polymerase chain reaction with sequence-specific primers (PCR–SSP) assay (PCR–SSP kit, Heidelberg University, Heidelberg, Germany) [25]. Amplification was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. When the quality of agarose gel was not acceptable, the result was excluded. After electrophoresis, the gel was placed on a UV transilluminator, and a picture for interpretation and documentation was taken. Each of the primer mixes contained a control primer pair that amplified either a part of the β-globin gene or a part of the C-reactive protein (CRP) gene. The β-globin control primers produce a 89-bp fragment, while the primer pairs amplifying the CRP gene produced a 440-bp amplicon. The allele and genotype frequencies of the following cytokine genes were determined: IL-2 G(−330)T and G(+166)T; IL-4 G(−1098)T, C(−590)T, and C(−33)T; IL-10 G(−1082)A, C(−819)T, and C(−592)A; TGF-β T(+869)C and G(+915)C; and IFN-γ A(UTR +5644)T.

Statistics

Data analysis was done using the Epi Info statistical software (version 6.2, World Health Organization, Geneva, Switzerland). Allele frequencies were estimated by direct gene counting. Allele frequencies of various genotypes were

Table 1. Allele Frequencies of IBS Patients in Comparison with Normal Controls

Cytokine	Position	Allele	Patients (n=70) N (%)	Controls (n=140) N (%)	P value[a]	Odds ratio (95% confidence interval)
IL-2	+166	G	97 (74.6%)	219 (78.8%)	0.417	0.79 (0.47–1.33)
		T	33 (25.38%)	59 (21.2%)		1.26 (0.75–2.12)
IL-2	−330	G	56 (43.07%)	110 (39.6%)	0.572	1.16 (0.74–1.80)
		T	74 (56.92%)	168 (60.4%)		0.87 (0.55–1.35)
IL-4	−1098	G	29 (23.77%)	84 (30.2%)	0.231	0.72 (0.43–1.21)
		T	93 (76.22%)	194 (69.8%)		1.39 (0.83–2.33)
IL-4	−590	C	86 (70.49%)	149 (53.6%)	0.002	2.07 (1.28–3.35)
		T	36 (29.5%)	129 (46.4%)		0.48 (0.30–0.78)
IL-4	−33	C	94 (77.04%)	200 (71.9%)	0.345	1.31 (0.78–2.22)
		T	28 (22.95%)	78 (28.1%)		0.76 (0.45–1.29)
IL-10	−1082	A	76 (57.57%)	181 (64.6%)	0.203	0.74 (0.48–1.16)
		G	56 (42.42%)	99 (35.4%)		1.35 (0.86–2.10)
IL-10	−819	C	96 (72.72%)	199 (71.1%)	0.817	1.09 (0.67–1.77)
		T	36 (27.27%)	81 (28.9%)		0.92 (0.57–1.50)
IL-10	−592	A	36 (27.48%)	81 (28.9%)	0.852	0.93 (0.57–1.52)
		C	95 (72.51%)	199 (71.1%)		0.07 (0.66–1.75)
IFN-γ	UTR +5644	A	78 (55.71%)	140 (50.7%)	0.390	1.22 (0.80–1.88)
		T	62 (44.28%)	136 (49.3%)		0.82 (0.53–1.26)
TGF-β	+869	C	51 (46.36%)	131 (47.5%)	0.934	0.96 (0.60–1.53)
		T	59 (53.63%)	145 (52.5%)		1.05 (0.66–1.67)
TGF-β	+915	C	4 (3.63%)	21 (7.6%)	0.229	0.46 (0.13–1.46)
		G	106 (96.36%)	255 (92.4%)		2.18 (0.69–7.70)

[a] Significant at 5% level adjusted for Bonferroni multiple comparison correction

compared using the chi-square test. The odds ratio and 95% confidence intervals (CI) were calculated for each allele/genotype/haplotype in the patient and control groups. Bonferroni correction method was utilized to adjust multiple comparisons according to α/n formula, where study-wide error rate (α) was equal to 0.05 and n was equal to the number of statistical comparisons for each set of data (alleles, genotypes, or haplotypes).

RESULTS

IL-4 Gene Polymorphism

The frequency of the IL-4 C allele at position −590 (70% in patients *vs.* 54% in controls, P=0.002) in the patient group was much higher than in the control group (Table 1). IL-4 CC genotype at position −590 (44% in patients *vs.* 7% in controls, P<0.001) and TT genotype at position −33 (10% in patients *vs.* 0% in controls, P< 0.001) in the patient group were significantly over-represented, while IL-4 TC genotype at position −590 (53% in patients *vs.* 93% in controls, P<0.001) and TC genotype at position −33 (26% in patients *vs.* 56% in controls, P<0.001) were significantly decreased in the patient group (Table 2). The frequencies of IL-4 (−1098, −590, −33) TCC haplotype (47% in patients *vs.* 23% in controls, P<0.001) and TCT haplotype (9% in patients *vs.* 1% in controls, P<0.001) in the IBS patients were significantly higher than in the control group, while the frequencies of the following haplotypes in the patient

Table 2. Genotype Frequencies of IBS Patients in Comparison with Normal Controls

Cytokine	Position	Genotypes	Patients (n=70) N (%)	Controls (n=140) N (%)	P value[a]	Odds ratio (95% confidence interval)
IL-2	+166	GG	36 (55.38%)	82 (59.0%)	0.738	0.86 (0.46–1.63)
		GT	25 (38.46%)	55 (39.6%)	0.997	0.95 (0.50–1.82)
		TT	4 (6.15%)	2 (1.4%)	0.583	4.49 (0.68–36.38)
IL-2	−330	GG	10 (15.38%)	8 (5.8%)	0.046	2.98 (1.02–8.82)
		GT	36 (55.38%)	94 (67.6%)	0.124	0.59 (0.31–1.14)
		TT	19 (29.23%)	37 (26.6%)	0.824	1.14 (0.56–2.30)
IL-4	−1098	GG	2 (3.27%)	1 (0.7%)	0.221	4.68 (0.32–133.01)
		TG	25 (40.98%)	82 (59.0%)	0.028	0.48 (0.25–0.93)
		TT	34 (55.73%)	56 (40.3%)	0.061	1.78 (0.97–3.59)
IL-4	−590	CC	27 (44.26%)	10 (7.2%)	<0.001	10.24 (4.24–25.30)
		TC	32 (52.45%)	129 (92.8%)	<0.001	0.09 (0.03–0.21)
		TT	2 (3.27%)	0 (0.0%)	0.092	–
IL-4	−33	CC	39 (63.93%)	61 (43.9%)	0.014	2.27 (1.17–4.43)
		TC	16 (26.22%)	78 (56.1%)	<0.001	0.28 (0.14–0.56)
		TT	6 (9.83%)	0 (0.0%)	<0.001	–
IL-10	−1082	AA	12 (18.18%)	53 (37.8%)	0.007	0.36 (0.17–0.78)
		GA	52 (78.78%)	75 (53.6%)	<0.001	3.22 (1.56–6.72)
		GG	2 (3.03%)	12 (8.6%)	0.233	0.33 (0.05–1.69)
IL-10	−819	CC	31 (46.96%)	71 (50.7%)	0.724	0.86 (0.46–1.61)
		CT	34 (51.51%)	57 (40.7%)	0.191	1.55 (0.82–2.91)
		TT	1 (1.515%)	12 (8.6%)	0.065	0.16 (0.01–1.26)
IL-10	−592	AA	1 (1.53%)	12 (8.6%)	0.066	0.17 (0.01–1.28)
		CA	34 (52.30%)	57 (40.7%)	0.160	1.60 (0.85–3.01)
		CC	30 (45.45%)	71 (50.7%)	0.467	0.83 (0.44–1.57)
IFN-γ	UTR +5644	AA	23 (32.85%)	43 (31.2%)	0.927	1.08 (0.56–2.59)
		AT	32 (45.71%)	54 (39.1%)	0.445	1.31 (0.70–2.44)
		TT	15 (21.42%)	41 (29.7%)	0.268	0.65 (0.31–1.33)
TGF-β	+869	CC	10 (18.18%)	20 (14.5%)	0.675	1.31 (0.52–3.23)
		CT	31 (56.36%)	91 (65.9%)	0.280	0.67 (0.34–1.33)
		TT	14 (25.45%)	27 (19.6%)	0.478	1.40 (0.63–3.12)
TGF-β	+915	CC	1 (1.8%)	2 (1.5%)	1.0000	1.26 (–)
		CG	2 (3.63%)	17 (12.3%)	0.118	0.27 (0.04–1.28)
		GG	52 (94.5%)	119 (86.2%)	0.164	2.77 (0.73–12.32)

[a] Significant at 5% level adjusted for Bonferroni multiple comparison correction

group were significantly lower than the controls: IL-4 (−1098, −590, −33) TTT haplotype (12% in patients *vs.* 27% in controls, P=0.002) (Table 3).

IL-10 Gene Polymorphism

The frequency of IL-10 GA genotype at position −1082 in the patient group (79%) was significantly higher than the control group (54%; P<0.001), while the frequency of AA genotype at the same position was significantly decreased (18% in patients *vs.* 38% in controls, P=0.007; Table 2). There was not any significant difference on genotypes of other positions and also on haplotypes between two groups of patients and controls.

Other Cytokine Gene Polymorphisms

There was not any significant difference on genotypes of other cytokine between patients and controls. The only significant finding was on IL-2 (−330, +160) GT haplotype which was significantly overrepresented in the patient group (39% in patients *vs.* 0.3% in controls, P=0.002; Table 3).

DISCUSSION

Cytokines are modulators for the immune response, which have an important role in the regulation of the immune and inflammatory response in intestine [12, 13]. While cytokine production could be affected by cytokine gene polymorphism [14, 15], we have genotyped a sample of IBS patients for a number of these cytokines.

IL-4 is an important cytokine in differentiation of naïve helper T-cells to T-helper 2 cells [26]. Three cytokine single-nucleotide polymorphisms (SNPs) situated at positions −1098 (G/T), −590 (C/T), and −33 (C/T) in the promoter region of IL-4 gene were investigated in this study.

The frequency of the IL-4 C allele at position −590 in the patient group was significantly higher than the control group. The frequencies of IL-4 CC genotype at the same position and IL-4 TT genotype at position −33 were also significantly increased in the patient group, while the frequencies of IL-4 TC genotype (−590) and IL-4 TC genotype (−33) were significantly decreased in this group of patients. The haplotypes GCC, TTT, TCC, and TTC are four common haplotypes in Iranian normal populations [25]. There was an increased frequency of IL-4 (−1098, −590, −33) TCC and TCT haplotypes and decreased frequency of TTT haplotype in the patient group. To our best knowledge, this is the first time that such associations between IL-4 gene polymorphisms and IBS are reported.

IL-10 is an important immunomodulatory cytokine, which was initially characterized as cytokine synthesis inhibitory factor that inhibits production of several cytokines

Table 3. Haplotype Frequencies of IBS Patients in Comparison with Normal Controls

Cytokine	Haplotypes	Patients (n=70) N (%)	Controls (n=140) N (%)	P value[a]	Odds ratio (95% confidence interval)
IL-2 (−330, +166)	GG	49 (24.25%)	107 (38.8%)	0.921	0.96 (0.61–1.50)
	TG	48 (23.76%)	112 (40.6%)	0.552	0.86 (0.55–1.35)
	TT	26 (12.87%)	56 (20.3%)	0.948	0.98 (0.56–1.70)
	GT	79 (39.10%)	1 (0.3%)	0.002	15.56 (1.91–342.11)
IL-4 (−1098, −590, −33)	GCC	21 (18.26%)	83 (30.0%)	0.024	0.52 (0.30–0.93)
	TTT	14 (12.17%)	76 (27.3%)	0.002	0.37 (0.19–0.71)
	TCC	54 (46.95%)	65 (23.4%)	<0.001	5.80 (3.61–9.33)
	TTC	14 (12.17%)	51 (18.3%)	0.177	0.62 (0.31–1.21)
	TCT	10 (8.69%)	2 (0.7%)	<0.001	13.14 (2.56–88.37)
	GTT	2 (1.73%)	1 (0.3%)	0.206	4.90 (0.35–137.92)
IL-10 (−1082, −819, −592)	ACC	36 (27.9%)	100 (35.7%)	0.136	069 (0.43–1.11)
	GCC	56 (43.41%)	99 (35.4%)	0.203	1.35 (0.86–2.10)
	ATA	33 (25.58%)	81 (28.9%)	0.475	0.82 (0.50–1.35)
	ACA	4 (3.1%)	0 (0.0%)	0.010	–
	ATC	3 (2.32%)	0 (0.0%)	0.034	–
TGF-β (+869, +915)	CG	45 (41.66%)	110 (39.9%)	0.834	1.08 (0.67–1.74)
	TG	58 (53.70%)	145 (52.5%)	0.926	1.05 (0.65–1.68)
	CC	4 (3.70%)	21 (7.6%)	0.244	0.47 (0.13–1.48)
	TC	1 (0.92%)	0 (0.0%)	0.380	–

[a] Significant at 5% level adjusted for Bonferroni multiple comparison correction

such as IFN-γ, IL-2, IL-4, IL-6, and TNF-α [27, 28]. Three SNPs situated at positions −1082 (G/A), −819 (C/T), and −592 (C/A) in the promoter region of IL-10 gene were investigated in this study. Statistical analysis of IL-10 gene polymorphisms at position −1082 revealed significantly higher frequency of GA genotype and lower frequency of AA genotype in the patients with IBS. Production of IL-10 is associated with SNPs at positions −1082 (G/A) and −819 (C/T) [29]. Although, in the study by Gonsalkorale *et al.* [30], a lower frequency of high producer IL-10 genotype (GG at −1082) was reported in the IBS patients, genotyping of IL-10 in the study by van der Veek *et al.* [11] revealed similar distribution in patients and controls. Frequency of high producer genotype (GG at −1082) was as low as 3% in the patient group and 8% in the control group, which is much lower than previous studies [11, 30] and could be due to various ethnic groups in different regions [31]. It seems that IL-10 AA genotype at −1082 is associated with low production of IL-10, whereas GA genotype at the same position is associated with intermediate production of IL-10 [29]. Therefore, considering decreased frequency AA genotype (low producer IL-10) and increased frequency of GA genotype (intermediate producer IL-10) in this study, an intermediate to high production of IL-10 is expected in the patients with IBS.

Genetic predisposition to produce high or low amounts of a particular cytokine could suggest a susceptible or protective role of that cytokine, respectively. Further studies on cytokine gene polymorphisms in IBS should be performed in different regions of the world to provide satisfactory evidence in the pathophysiology of the disease.

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