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Association of Interleukin 10 and Transforming Growth Factor β Gene Polymorphisms with Chronic Idiopathic Urticaria

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SUMMARY Transforming growth factor β (TGF-β) and interleukin 10 (IL-10) are two anti-inflammatory cytokines that are implicated in the pathogenesis of urticaria. The goal of this study was to examine the possible association of polymorphisms of TGF-β and IL-10 genes with susceptibility to chronic idiopathic urticaria (CIU). This study was conducted on 90 patients with CIU. Polymerase chain reaction (PCR) was done to determine the genotype at 5 polymorphic sites; TGF-β (codon10C/T and codon25G/C) and IL-10 (-1082G/A, -819C/T, and -592C/A). The C allele at codon 25 of TGF-β was more prevalent in CIU patients compared to controls (OR = 9.5, 95% CI = 5.4-16.8, *P*<0.001). Genotypes of CT and CG at 10 and 25 codons of TGF-β gene, respectively, and AG, CT, and CA for loci of -1082, -819, and -592 of IL-10 gene were significantly higher in CIU patients (*P*<0.001). In haplotype analysis, frequency of TGF-β haplotypes differed between patients with CIU and controls; CC haplotype was overrepresented, while CG and TG haplotypes were underrepresented (*P*<0.001). These results suggest that TGF-β and IL-10 genetic variability could contribute to susceptibility to CIU. Additionally, patients with CIU seem to have genotypes leading to high production of TGF-β and IL-10.

KEYWORDS: interleukin 10 (IL-10); transforming growth factor β (TGF-β); chronic idiopathic urticaria; single nucleotide polymorphism (SNP)

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INTRODUCTION

Chronic idiopathic urticaria (CIU) is characterized by itching wheals lasting for more than six weeks with unknown etiology. The pathophysiology of CIU is complex, and the diagnosis is made when no identifiable cause has been detected for pruritic lesions (1,2). Mast cell activation has been suggested to be the pivotal mechanism behind CIU. Histamine and leukotrienes mediate inflammation and cause persistence of cutaneous wheals. For initiation and maintenance of the inflammatory process, cytokines are the key factor. Cytokines are implicated not only in recruitment of inflammatory cells to the target site, but also in triggering the cell signaling that leads to release of histamine and eicosanoids (3,4).

Mast cell activation leads to not only the release of histamine and leukotrienes, but also production of proinflammatory cytokines that promote inflammatory response (5). Previously, our research group demonstrated that polymorphisms in promoter region of proinflammatory cytokines are associated with CIU (6). On the other hand, regulatory mechanisms can suppress the inflammatory process by inhibition of either mast cells or other cell types contributing to the inflammation. Transforming growth factor β1 (TGF-β1) has been demonstrated to play an important role in homeostasis of mast cells (7). TGF-β1 promotes apoptosis of mast cells (8), suppresses expression of both of the Fc-epsilon receptor I (FcεRI) and immunoglobin E (Ig-E) on mast cell surface (9), and attenuates mediator release from mast cells (8). Interleukin 10 (IL-10), another anti-inflammatory cytokine, exerts similar effects such as inhibition of expression of FcεRI on mast cells and suppression of inflammatory mediator release (10, 11). Furthermore, IL-10 is able to induce apoptosis of not only mast cells, but also other inflammatory cells (12). It has been shown that patients with CIU have decreased serum levels of IL-10, compared to healthy controls (13). On the other hand, some patients with CIU showed significantly increased levels of IL-10 in cutaneous samples or sera compared with the healthy population (8-12). Alteration in cytokine profile of patients with CIU may be of importance in the pathophysiology of CIU.

Reduced levels of anti-inflammatory cytokines may be caused by reduced expression of cytokine genes. Transcription initiation is the main step of gene expression regulation, and polymorphisms at the promoter region may lead to altered gene expression (14). We hypothesized that genetic variability in the promoter region of IL-10 as well as polymorphisms in the coding region of TGF-β could contribute to the risk of CIU. Accordingly, in this study we aimed to investigate allele frequencies, genotypes, and haplotypes of TGF-β and IL-10 in patients with CIU in comparison with controls.

PATIENTS AND METHODS

Participants and study design

This hospital-based case-control study was comprised of 90 patients with CIU. Diagnosis of CIU was made based on international guidelines, thus including patients with at least 2 occurrences of wheals for more than 6 weeks in the absence of any identifiable cause (15). The patients were randomly selected from the outpatient clinic of allergy and immunology in the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. One hundred and forty healthy subjects were also randomly selected (16). A detailed history and physical examination were taken prior to enrolment to ensure that all participants met the selection criteria. Subjects with physical urticaria, cold urticaria, urticaria vasculitis, and drug induced urticaria were excluded from the patient group. Subjects who were suspected to have chronic urticaria, allergic disease, or autoimmunities were not included as controls. No matching was done

Table 1. Allele frequency of TGF-β and IL-10 polymorphic sites in patients with CIU as compared with controls

Cytokine	Position	Alleles	Controls	CIU	OR (95% CI)	*P* value
TGF-β	Codon 10	C	131 (47.5)	83 (46)	1 (ref)	
		T	145 (52.5)	97 (53.8)	1.1 (0.7-1.6)	0.85
TGF-β	Codon 25	G	255 (92.4)	101 (56.1)	1 (ref)	
		C	21 (7.6)	79 (43.8)	9.5 (5.4-16.8)	<0.001
IL-10	-1082	A	181 (64.6)	101 (56.7)	1 (ref)	
		G	99 (35.4)	77 (43.2)	1.39 (0.93-2.09)	0.11
IL-10	-819	C	199 (71.1)	116 (65.9)	1 (ref)	
		T	81 (28.9)	60 (34)	1.27 (0.83-1.94)	0.29
IL-10	-592	C	199 (71.1)	120 (67.4)	1 (ref)	
		A	81 (28.9)	58 (32.6)	1.19 (0.77-1.82)	0.47

Frequencies are expressed as number (percent).

in terms of age and gender, but all the participants had the same ethnicity. This study was approved by the Institutional Review Board of Tehran University of Medical Sciences. All of the participants received detailed information regarding the aim and protocol of study and signed informed consent forms.

DNA extraction and genotyping

Blood samples were taken from all of the participants and preserved with Ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant. Genomic DNA was isolated from peripheral blood leukocytes using phenol chloroform extraction. Polymerase chain reaction (PCR) with sequence specific primers was used for genotyping (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as explained before (16). PCR products were detected with 2% agarose gel electrophoresis and visualized using an ultraviolet (UV) transilluminator. The polymorphic sites tested in this study included TGF-β [codon10 or +869C/T (rs1982073) and codon25 or +915G/C (rs1800471)] and IL-10 [-1082G/A (rs1800896), -819C/T (rs1800871), -592C/A (rs1800872)].

Statistical analysis

All of the analyses were performed using the Epi Info statistical software (version 6.2, World Health Organization, Geneva, Switzerland). Required sample size was calculated according to previously published methods with α = 0.05, β = 0.2 (power = 80%) and the ratio of 3:2 for controls:cases (17). Direct gene counting was used to estimate allele, genotype, and haplotype frequencies. Estimated frequencies were compared between patients with CIU and controls using the chi square test or Fisher's exact test as appropriate. A 95% confidence interval (95% CI) was estimated for allele, genotype, and haplotype frequencies in patients with CIU as compared with controls. To adjust 95% CIs for sex, the Bonferroni method was used. All the tests were two sided, and probability of less than 0.05 was considered significant.

RESULTS

Allele analysis

All of the allele frequencies are in line with the Hardy-Weinberg equilibrium. Among the tested polymorphic sites, frequency of C allele at codon 25 of the TGF-β gene was significantly higher in patients with CIU, as compared to controls (43.8% versus 7.6%, $P<0.001$). Other allele frequencies did not differ between CIU patients and healthy controls (Table 1).

Genotyping results

Genotype analysis revealed that the following genotypes were overrepresented in patients with CIU: TGF-β genotypes of CT and CG for 10 and 25 codons and IL-10 genotypes of AG, CT, and CA for loci of -1082, -819, and -592, respectively ($P<0.001$). TT at codon 10 of TGF-β, CC at codon25 of TGF-β, and AG at -1082, CT at -819, and AA at -592 in promoter of IL-10 were significantly lower in CIU patients. Patients with CIU were more heterozygous for evaluated genotypes. Detailed frequencies regarding each genotype are outlined in Table 2.

Table 2. Genotype frequency of TGF-β and IL-10 polymorphic sites in patients with CIU as compared with controls

Cytokine	Position	Alleles	Controls	CIU	OR (95% CI)	*P* value
TGF-β	Codon 10	CC	20 (14.5)	0	1 (ref)	
		CT	91 (65.9)	83 (92.2)	1.1 (0.7-1.6)	<0.001
		TT	27 (19.6)	7 (7.7)	0.35 (0.13-0.89)	0.02
TGF-β	Codon 25	GG	119 (86.2)	11 (12)	1 (ref)	
		GC	17 (12.3)	79 (87.7)	51.1 (21.3-126)	<0.001
		CC	2 (1.5)	0	0.0 (0.0-6.3)	<0.001
IL-10	-1082	AA	53 (37.8)	16 (17.9)	1 (ref)	
		AG	75 (53.6)	69 (77.5)	2.99 (1.58-5.69)	<0.001
		GG	12 (8.6)	4 (4.42)	0.50 (0.13-1.75)	0.36
IL-10	-819	CC	71 (50.7)	30 (34)	1 (ref)	
		CT	57 (40.7)	56 (63.6)	2.55 (1.42-4.59)	0.001
		TT	12 (8.6)	2 (2.27)	0.25 (0.04-1.21)	0.09
IL-10	-592	CC	71 (50.7)	32 (35.9)	1 (ref)	
		CA	57 (40.7)	56 (62.9)	2.47 (1.38-4.43)	0.002
		AA	12 (8.6)	1 (1.12)	0.55 (0.30-0.97)	0.04

Frequencies are expressed as number (percent).

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Table 3. Haplotype frequency of TGF-β and IL-10 polymorphic sites in patients with CIU as compared with controls

Cytokine	Haplotype	Controls	CIU	OR (95% CI)	*P* value
TGF-β (codon 10G/A and 25G/C)	CG	110 (39.9)	83 (25.46)	0.5 (0.4-0.7)	<0.001
	TG	145 (52.5)	90 (27.6)	0.3 (0.2-0.5)	<0.001
	CC	21 (7.6)	74 (22.69)	3.6 (2.1-6.2)	<0.001
	TC	0 (0)	79 (24.2)	Reference	-
IL-10 (-1082G/A, -819C/T and -592C/A)	GCC	99 (35.4)	73 (35.6)	1.0 (0.7-.5)	0.97
	ACC	100 (35.7)	82 (40)	1.2 (0.8-.8)	0.38
	ATA	81 (28.9)	50 (24.4)	Reference	-

Frequencies are expressed as number (percent).

Haplotype analysis

None of the possible haplotypes of ACA, GCA, ATC, GTC, and GTA of IL-10 polymorphic sites of -1082, -819, and -592 were observed in participants. GCC, ACC, and ATA haplotypes for IL-10 polymorphic sites were equally distributed between patients with CIU and controls. All of the possible haplotypes of TGF-β (C/T at codon 10 and G/C at codon 25) significantly differed in CIU patients as compared with controls; CC haplotype was overrepresented, while CG and TG haplotypes were underrepresented. Detailed haplotype analysis is outlined in Table 3.

DISCUSSION

CIU patients suffer from recurrent chronic urticarial lesions without any specific cause. Genetic predisposition may be of high importance in development of CIU, and our study is the first to show the association of the TGF-β coding region and IL-10 promoter polymorphisms with CIU. The results of this study provide evidence that genetic variability in TGF-β and IL-10 genes contributes to susceptibility to CIU. Two polymorphic sites in the TGF-β gene [codon10C/T at +869 (rs1982073) and codon25G/C at +915 (rs1800471)] and three polymorphic sites in promoter region of IL-10 gene [-1082G/A (rs1800896), -819C/T (rs1800871), -592C/A (rs1800872)] were investigated in patients with CIU and healthy subjects as controls. The strongest association was found in codon25G/C (rs1800471) in the coding sequence of TGF-β gene on chromosome 19q13.1. Carriers of minor C allele and GC genotype were significantly higher in patients with CIU, with OR of 9.5 (95% CI, 5.4-16.8) and 51.1 (95% CI, 21.3-126), respectively. These polymorphisms in the TGF-β first exon lead to substitution of leucine (T) with proline (C) at codon10 and arginine (G) for proline (C) at codon25. It has been suggested that this genetic variability may affect TGF-β production; GG genotype at rs1800471 (+915G/C) in combination with TT genotype at rs1982073 (+869T/C) result in higher production of TGF-β (18). Another study on patients with gastric cancer demonstrated that carriers of GC genotype at rs1800471 had the highest serum level of TGF-β as compared to other genotypes (19). CC genotype is associated with the lowest production of TGF-β, whether at rs1800471 or 1982073 polymorphic sites, while GG genotype leads to the high TGF-β production. Homozygous genotype of CC that leads to low TGF-β1 production and homozygous genotypes of GG or TT leading to high production of TGF-β1 were significantly lower in patients with CIU; No patients with CIU had CC genotype; GG genotype at rs1800471 was found only in 12% of patients with CIU as compared with 86.2% of controls; TT genotype at rs1982073 was found in 7.7% of patients with CIU as compared with 19.6% of healthy subjects ($P<0.001$). As with the higher carriage of C allele in patients with, heterozygous genotypes that are associated with high TGF-β1 production were significantly higher in patients with CIU. In interpreting these findings regarding TGF-β polymorphisms, ethnicity cannot be an affecting factor because a previous study on Iranians showed that the minor C allele was only present in 7-10% of population without any significant differences form Caucasians from Greece, England, and Finland and African-Americans (16).Participants in our study were in the same age-range and ORs were adjusted with respect to gender. Controls were healthy, and patients with CIU were free of any comorbidity such as autoimmunity, cancer, or chronic disease that could be considered a confounder. Hence, the significant association of TGF-β polymorphism with CIU seems not to be affected with any confounding factor.

Park *et al.* demonstrated that promoter polymorphism of TGF-β -509C/T is associated with aspirin intolerant chronic urticaria development. In that study in Korean patients, TT genotype frequencies were significantly higher in aspirin intolerant patients with

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chronic urticarial, whereas TGF-β1 serum levels tended to be lower (20). In patients with atopic dermatitis, Arkwright *et al.* (21) found that among IL-10 -1082G/A (rs1800896), TGF-β codon10C/T (rs1982073), and TGF-β (rs1800471) codon25G/C polymorphic sites, only C allele at rs1800471 was significantly higher in patients (OR = 4.8, 95% CI = 2.4-9.7). In our findings, patients with CIU also had higher C allele frequencies but with greater OR of 9.5 and 95% CI of 5.4 to 16.8. Our study also found higher frequency of GC genotype in patients with CIU that is consistent with the study by Arkwrigh *et al.* (21) in which 40% of patients with atopic dermatitis had GC genotype as compared with 12% of controls (*P*=0.001). GC genotype at codon25G/C (rs1800471) was previously demonstrated to be higher in patients with asthma (22) and common variable immunodeficiency (23) in Iranian population. Inappropriate immune response and imbalance in cytokine profile are implicated in both the diseases.

The TGF-β gene encodes TGF-β1 that belongs to the TGF-β superfamily. TGF-β1 is a cytokine with diverse effects on cell function, differentiation, and proliferation. TGF-β1 also plays a crucial role in skin biology; TGF-β1 regulates homeostasis of keratinocytes, antigen presenting cells, and inflammatory cells. TGF-β1 is implicated in cutaneous wound healing (24), clearance of apoptotic cells from skin tissue (25), maintenance of a healthy skin barrier function (26), and interference with neoplastic cell adhesion and growth in skin (27). Additionally, TGF-β1 had contrasting roles in development of skin hypersensitivity. Over-expression of TGF-β1 by keratinocytes, as well as exogenous addition of TGF-β to culture of skin explants, leads to induction of inflammation, infiltration of plasmacytoid dendritic cells to dermis (28), and increase in eotaxin production by dermal fibroblasts (29). On the other hand, Smad3-/- mice that lack intracellular signaling mediator of TGF-β showed increased expression of proinflammatory and T-helper2 cytokines and developed contact hypersensitivity (30). TGF-β1 also acts as an anti-inflammatory cytokine in skin mainly via inhibition of mast cell survival and function (9,31,32). However, the pathophysiology of CIU has not been well defined. Mast cells seem to be pivotal factors in CIU (1,3). Mast cells are major cells responsible for histamine release and urticarial lesion formation in CIU. Increase in mast cell release, especially during active rather than remission stage of CIU, is commonly seen. Ferrer *et al.* demonstrated that CIU patients have higher serum tryptase levels compared with healthy controls and even atopic patients free of CIU. They also found that this marker of mast cell degranulation is increased during the active stage of CIU (33). Anti-histamines help relieve the symptoms in these patients; however, some patients do not respond adequately to this treatment, indicating complexity of CIU pathogenesis (1,3). Immunoregulatory cytokines such as TGF-β1 and IL-10 inhibit T-helper2 cytokines as well as mast cell survival, FCεRI expression, binding to Ig-E, and mediator release from mast cells (8-12, 32).

IL-10 is an anti-inflammatory cytokine that is secreted by macrophages and T lymphocytes. IL-10 inhibits proinflammatory cytokine production and has inhibitory effects on mast cells (34). It has been previously shown that patients with CIU have lower serum levels of IL-10 compared with the healthy population (13). By contrast, some studies showed significantly increased levels of IL-10 in cutaneous samples or in the sera of patients with CIU compared with the healthy population (8-12,32). It is not yet understood whether this increase in IL-10 is a consequence or compensatory response. Polymorphisms in the IL-10 gene (located on chromosome 1q31-32) can affect expression of IL-10. Changes in promoter region sequence alter the affinity of proteins involving in transcription and finally alter the level of gene product. All reported polymorphic sites in the promoter of IL-10 were investigated in participants of our study. No significant difference was found in terms of allele and haplotype frequencies in patients with as compared with controls, while, heterozygous genotypes were significantly higher in all loci in the patient group. Additionally, only GCC, ATA and ACC were observed in participants out of all possible haplotypes. This may be explained by a strong linkage disequilibrium that is in contrast with TGF-β polymorphic sites in which linkage disequilibrium is incomplete (35). Alleles of G at -1082G/A, T at -819C/T, and A at -592C/A are associated with higher production of IL-10 in comparison with their counterparts (36). All aforementioned alleles were higher in patients with CIU in our study, but these differences were not statistically significant. This is an unexpected finding, since we expected to observe higher frequency of low producing alleles and genotypes in patients with CIU. Interestingly, Palikhe *et al.* found that T allele at -819C/T is higher in aspirin intolerant patients with chronic urticaria (37). Picconi *et al.* (38) found that levels of IL-10 as well as tumor necrosis factor-α, macrophage inflammatory protein-1α and chemokine (C-C motif) ligand-5 (also CCL5) were higher in CIU patients, while IL-2 and interferon-γ were reduced. They suggested increased level of IL-10 could be a secondary phenomenon hampering the inflammation. However, the results of our study indicate that patients with CIU have a high IL-10 producing genetic background, and increased

levels of IL-10 would be a primary rather than a compensatory phenomenon in CIU. Accordingly, the authors suggest conducting further studies on patients with CIU to elucidate theinterplay of different groups of cytokines in the development of the disease.

Some studies suggested that IL-10 polymorphism frequencies may be affected by ethnic differences, since C allele at -819 was the most common allele in Asians, while in Caucasians from Europe T was the most common allele (39). Iranians showed similar allele frequencies as Caucasians at all polymorphic sites of the IL-10 gene (16).Furthermore, Palikhe *et al.* (37) found that the T allele at -819C/T was the most common allele in the Korean population. Thus, ethnic differences could not have affected the results of our study. The study benefited from proper design (case-control) and power of 80% to find the association between genetic polymorphism in immunoregulatory cytokine genes with CIU. All the participants were unrelated and of the same ethnicity, and no deviation from Hardy-Weinberg equilibrium was observed. However, a limiting factor was the absence of measurement of IL-10 and TGF-β1 levels in serum or gene expression using luciferase assay.

CONCLUSION

Further studies are needed to draw firmer conclusions regarding the genetic background of patients with CIU and the role of immunoregulatory cytokines in CIU.

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