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Original article

Interleukin-10 and *Transforming Growth Factor Beta1* Gene Polymorphisms in Chronic Heart Failure

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Summary. *Background:* As cytokines, including interleukin-10 (IL-10) and transforming growth factor beta 1(TGF-β1) seem to contribute towards the pathogenesis of chronic heart failure (CHF), this study was performed to assess the associations of certain single nucleotide polymorphisms (SNPs) of these genes in a case control study. *Methods:* This investigation was carried out to determine the frequency of alleles, genotypes and haplotypes of *TGF-β1* and *IL-10* single-nucleotide polymorphisms (SNPs) in 57 Iranian patients with CHF compared with 140 healthy subjects using polymerase chain reaction with sequence-specific primers method. **Results:** Results of the analyzed data divulged a negative association for both *TGF-β1* GC genotype at codon 25 (*P*=0.047) and CT genotype at codon 10 (*P*=0.018) and CHF proneness. Although, *TGF-β1* CC genotype at codon 10 was found to be positively associated with CHF (*P*=0.011). Moreover, the frequency of *IL-10* (–1082, -819, -592) ATA haplotype and *TGF-β1* (codon 10, codon 25) TG haplotype were significantly lower in the patients group (*P*=0.004 and *P*=0.040, respectively), while *TGF-β1* (codon 10, codon 25) CG haplotype was overrepresented in patients with CHF (*P*=0.007). *Conclusions:* Cytokine gene polymorphisms might affect vulnerability to CHF. Particular genotypes and haplotypes in *IL-10* and *TGF-β1* genes could render individuals more susceptible to CHF. (www.actabiomedica.it)

Key words: heart failure; single nucleotide polymorphism; interleukin-10; transforming growth factor beta1

Introduction

Chronic heart failure (CHF) is an intricate public health problem, characterized by impaired contractile function and gradual ventricular dilation (1). It has been understood that several physiologic systems, including the immune system, engage in the pathogenesis of this complex multi-step disease (2). Considering high morbidity and mortality of CHF despite utilizing current treatment modalities, it stands to reason that identification of gene variations affecting underlying pathogenic mechanisms, seems necessary to improve the disease treatment strategies.

CHF is characterized by systemic inflammation, as evident by elevated circulating levels of multiple inflammatory cytokines with increasing levels in ac-

cordance with the extent of disease severity (3).Cytokines have been also implicated in the pathogenesis of underlying cardiovascular disorders such as atherosclerosis (4). Interleukin-10 (IL-10) is a significant immunoregulatory cytokine which exerts potent immunosuppressive functions by down-regulating the expression of co-stimulatory molecules and T helper 1 (TH1) cytokines (5). The other key immunoregulatory cytokine is transforming growth factor-beta1 (TGF-β1), to which certain vasculoprotective properties, comprising inhibition of the adhesion of neutrophils and T cells to the endothelium, transmigration of neutrophils through the endothelium, and production of pro-inflammatory adhesion molecules within endothelial cells, have been attributed (6-9).

It has been indicated that genetic polymorphisms within coding and promoter sequences of cytokine genes could modulate their production (10, 11). The association of certain cytokine gene polymorphisms and a number of diseases with possible underlying immune disturbances have already been studied (2, 12-21), whilst our understanding in CHF is restricted due to the scantiness of studies in this area. To the best of our knowledge, this is the first study exploring possible contributions of SNPs in *IL-10* and *TGF-β1* genes toward individual vulnerability to CHF in Iranian cases.

In order to evaluate the associations between the SNPs in *IL-10* gene at positions -1082, -819 and -592 and *TGF-β1* gene at codon 10 and codon 25 and CHF, this study was conducted in a group of Iranian patients and compared with healthy control subjects.

Patients and Methods

Subjects

In the current study, we investigated a total of 57 Iranian patients with chronic heart failure (43 male, 14 female) with the mean age 57.96±12.24. The control group is consisted of one hundred and forty unrelated individuals (mean age 45.63±10.84; 101 men, 39 women) who were randomly selected from healthy volunteers, as previously described (22). The diagnosis of chronic heart failure was based on thorough history taking, comprehensive physical examination, electrocardiography and impaired left ventricular (LV) systolic function (LV ejection fraction ≤40%) and LV dilation (LV end-diastolic diameter >5.5 cm) on echocardiography. We excluded all subjects with chronic lung disease, recent myocardial infarction, malignancies and acute decompensated HF within 3 months prior to enrollment. All the cases who fulfilled the inclusion criteria were in stable clinical condition and received conventional medical therapy for at least 3 months. Baseline clinical characteristics of patients with CHF are depicted in Table 1.

Written informed consents were taken from all participants before recruitment. This investigation was conducted according to the guidelines of the Ethics Committee of Tehran University of Medical Sciences.

Genotyping

For all of the entrants to the present study, amount of 5 milliliters (ml) of venous blood samples were obtained and kept with ethylenediaminetetraacetic acid

Table 1. Baseline clinical characteristics of patients with chronic heart failure

Characteristics	N (%)
Hypertension	21 (36.8%)
Diabetes	19 (36.8%)
Dyslipidemia	22 (38.6%)
Obesity	8 (14%)
History of smoking	
Current smoker	25 (43.9%)
Ex-smoker	4 (7%)
Non-smoker	28 (49.1%)
History of ACS	31 (54.4%)
Chronic kidney disease	5 (8.8%)
CVA	1 (1.8%)
History of CABG	5 (8.8%)
History of PCI	4 (7%)
NYHA classification	
I	15 (26.3%)
II	18 (31.6%)
III	15 (26.3%)
IV	9 (15.8%)

ACS acute coronary syndrome, CVA cerebrovascular accident, CABG coronary artery bypass grafting, PCI percutaneous coronary intervention, NYHA New York Heart Association

(EDTA) at -20°C until being investigated. Genomic DNA was extracted using the "salting out" technique (23). Cytokine typing was carried out on genomic DNA by polymerase chain reaction with sequence -specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as previously elucidated in detail (22). Briefly, amplification was performed using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The availability of PCR products was visualized by 2% agarose gel electrophoresis.

We have determined the allele and genotype frequencies of *TGF-β1* (C/T at codon 10; rs1800470, and C/G at codon 25; rs1800471) and *IL-10* (A/G at -1082; rs1800896, C/T at -819; rs1800871, and A/C at -592; rs1800872) genes.

Statistical Analysis

Allele, genotype, and haplotype frequencies for all cytokine gene polymorphisms were calculated by direct counting and compared with the controls using both Fisher's exact test and *chi square* test. The frequencies of different genotypes were compared using the chi-square test so as to test the Hardy-Weinberg equilibrium. The odds ratio (OR) and 95% confidence intervals were estimated. The *P* value of less than 0.05 was considered to be statistically significant.

Results

Alleles and Genotype Frequencies

We observed a higher frequency of heterozygous GC in *TGF-β1* at codon 25 in controls compared to CHF cases (12.3% in controls *versus* 2.2% in patients, *P*=0·047). Moreover, heterozygous CT in *TGF-β1* at codon 10 was found to be more frequent in healthy controls compared to patients with CHF. The frequency of heterozygous CT at codon 10 reached 65.9 and 46% in these groups, respectively (*P*=0·018). However, the prevalence of homozygous CC in *TGF-β1* at codon 10 was lower in controls than in patients (14.5% in controls *versus* 32% in patients, *P*=0·011). Although the frequencies of *TGF-β1* TT genotype at codon 10 together with CG genotype at codon 25 were similar in patients and controls groups.

The allele and genotype frequencies of *IL-10* at positions -592, -819 and -1082 as well as the allelic frequency of *TGF-β1* at codon 10 and codon 25 were similar in two groups of patients and controls.

Allelic and genotype frequencies in patients with chronic heart failure and healthy subjects are shown in Table 2.

Haplotype Frequencies

IL-10 ATA haplotype at positions -1082, -819 and -592 was found to be more frequent in healthy controls in comparison with patients group (28.9% in controls *versus* 15.2% in patients, *P* = 0·004). Furthermore, a positive association was detected between *TGF-β1* CG haplotype at codon 10 and codon 25 and individual susceptibility to CHF (56.7% in patients *versus* 39.9% in controls, *P*=0·007), while *TGF-β1* TG haplotype at the same positions was significantly lower than controls (40% in patients *versus* 52.5% in controls, *P*=0·04).

We observed no significant differences between the two groups neither for ACC and GCC haplotypes at positions -1082, -819 and -592 of *IL-10* gene nor for CC and TC haplotypes at codon 10 and codon 25 of *TGF-β1* gene.

Haplotype frequencies in patients with chronic heart failure and healthy subjects are depicted in Table 3.

Discussion

Heart failure may results from a variety of underlying disorders, including ischemic heart disease, dilated cardiomyopathy and hypertension (24). Current thinking promotes the notion that multiple inflammatory elements intervene with hemostatic factors and endothelium, resulting in plaque formation, and in this way, these factors contribute towards the pathogenesis of heart failure. These inflammatory proteins, comprising IL-6 and C-reactive protein, take action through different mechanisms, one of which is down-regulation of atheroprotective cytokines, namely IL-10 and

Table 2. *IL-10* and *TGF-β1* allele and genotype polymorphisms in Iranian patients with CHF and healthy controls

Cytokine	Position	Alleles/Genotypes	Patients (N=57)	Controls (N=140) N (%)	Odds Ratio (95% CI) N (%)	p-value
			N=138		**N=50**	
TGF-β1	Codon 10	C	131 (47.5)	55 (55)	1.35 (0.85-2.14)	0.202
		T	145 (52.5)	45 (45)		
		CC	**20 (14.5)**	**16 (32)**	**2.78 (1.3-5.94)**	**0.011**
		CT	**91 (65.9)**	**23 (46)**	**0.44 (0.23-0.85)**	**0.018**
		TT	27 (19.6)	11 (22)	1.16 (0.53-2.56)	0.687
			N=138	**N=46**		
TGF-β1	Codon 25	C	21 (7.6)	3 (3.3)	0.41 (0.12-1.41)	0.221
		G	255 (92.4)	89 (96.7)		
		CC	2 (1.5)	1 (2.2)	1.51 (0.13-17.06)	1
		GC	**17 (12.3)**	**1 (2.2)**	**0.16 (0.02-1.22)**	**0.047**
		GG	119 (86.2)	44 (95.6)	3.51 (0.79-15.7)	0.108
			N=140	**N=57**		
		A	181 (64.6)	75 (65.8)	1.05 (0.66-1.66)	0.907
		G	99 (35.4)	39 (34.2)		
			N=110	**N=54**		
IL-10	-1082	AA	23 (40.3)	20 (33.8)	1.11 (0.59-2.08)	0.750
		GA	75 (53.6)	29 (50.9)	0.9 (0.48-1.66)	0.755
		GG	12 (8.6)	5 (8.8)	1.02 (0.34-3.05)	1
			N=140	**N=56**		
		C	199 (71.1)	74 (66.1)	0.79 (0.49-1.27)	0.333
		T	81 (28.9)	38 (33.9)		
IL-10	-819	CC	71 (50.7)	26 (46.4)	0.84 (0.45-1.57)	0.637
		CT	57 (40.7)	22 (39.3)	0.94 (0.5-1.77)	0.873
		TT	12 (8.6)	8 (14.3)	1.78 (0.68-4.62)	0.295
			N=140	**N=57**		
		A	81 (28.9)	26 (22.8)	0.72 (0.44-1.21)	0.261
		C	199 (71.1)	88 (77.2)		
IL-10	-592	AA	12 (8.6)	2 (3.5)	0.39 (0.08-1.79)	0.358
		CA	57 (40.7)	22 (38.6)	0.91 (0.49-1.72)	0.873
		CC	71 (50.7)	33 (57.9)	1.34 (0.72-2.49)	0.432

Table 3. IL-10 and TGF-β1 haplotype polymorphisms in Iranian patients with CHF and healthy controls

Cytokine	Position	Haplotype	Controls (n=140) N (%)	Patients (n=57) N (%)	Odds Ratio (95% CI)	p-value
TGF-β1	Codon10, Codon25	**CG**	**110 (39.9)**	**51 (56.7)**	**1.97 (1.22-3.19)**	**0.007**
		TG	**145 (52.5)**	**36 (40)**	**0.6 (0.37-0.98)**	**0.040**
		CC	21 (7.6)	2 (2.2)	0.28 (0.06-1.2)	0.08
		TC	0 (0)	1 (1.1)	-	-
IL-10	-1082, -819, -592	GCC	99 (35.4)	34 (30.3)	0.8 (0.5-1.28)	0.409
		ACC	100 (35.7)	33 (29.5)	0.75 (0.47-1.21)	0.288
		ATA	**81 (28.9)**	**17 (15.2)**	**0.44 (0.25-0.78)**	**0.004**

TGF-β1 (25). While cytokine production could be regulated by gene polymorphisms (26), we have evaluated the involvement of certain functional single nucleotide polymorphisms within *IL-10* and *TGF-β1* genes in CHF susceptibility.

TGF-β1 is a multifunctional cytokine participating in several physiological and pathological processes. Multiple mechanisms have been suggested through which TGF-β1 exerts its effects on cardiovascular pathophysiology. These mechanisms include interfering with the development of atherosclerosis, influencing endothelial function, along with affecting vascular and cardiac remodeling to name but a few (27). In particular, elevated levels of serum or plasma TGF-β1 have been reported in patients with dilated cardiomyopathy or hypertension (28). In the present study, we evaluated two cytokine single-nucleotide polymorphisms situated at codon 10 (T869C, rs1982073) and codon 25 (G915C, rs1800471) in the coding region of *TGF-β1* gene. These gene variants have been proven to be associated with the levels of cytokine production (29). It has been postulated that *TGF-β1* CC and CT genotypes at codon 10, as well as *TGF-β1* GG and GC genotypes at codon 25 would be associated with higher TGF-*β*1 production level (30). At the genotype level, we detected down-regulation of both *TGF-β1* CT genotype (codon 10) together with GC genotype (codon 25) in addition to notable overexpression of codon 25 for the CC genotype in our patients group. Therefore, TGF-β1 could act as a protective factor against CHF in Iranian population, as the low-producing *TGF-β1* genotypes have been associated with CHF in our study. The frequency of *TGF-β1* (codon 10, codon 25) TG haplotype was significantly decreased in our group of patients, whilst CG haplotype was overrepresented in patients with CHF. In a recent meta-analysis of the role of *TGF-β1* gene polymorphisms in relation to the CHD risk, it was suggested that minor allele carriers of rs1800469 and rs1982073 genetic variants in *TGF-β1* have a 15% increased risk of CHD, although no significant association was observed between rs1800471 variant and CHD susceptibility (31). The other meta-analysis of the possible contributions of *TGF-β1* gene variants towards the development of CHD complications, such as myocardial infarction, indicated the association of rs180047 C allele with CHD complications (32).

IL-10 is a potent anti-inflammatory cytokine with pleiotropic effects in inflammation and immunoregulation. It diminishes the expression of MHC class 2 antigens, TH1 cytokines as well as co-stimulatory molecules on macrophages. Additionally, it upregulates B cell survival, proliferation and antibody production (33). It has been speculated that IL-10 protects endothelial function following an inflammatory stimulus via restricting superoxide synthesis within the vascular wall (34). The production of IL-10 is modified through a promoter region containing three SNPs situated at positions –1082 (G/A), –819 (C/T) and –592 (C/A) upstream from the transcriptional start site (35). Presence of the A allele at –592 has been related to low IL-10 production. Moreover, presence of an A allele at position –1082 has been correlated with a low IL-10 production by T lymphocytes as compared to a G allele (35). It has been previously demonstrated by Edwards-Smith et al. that the *IL-10* promoter haplotypes (–1082, –819, and –592) ATA, ACC, and GCC were associated with low, intermediate, and high IL-10 production, respectively (36). In the current study, we investigated these three SNPs in both patients and controls groups. Statistical analysis of *IL-10* gene polymorphisms disclosed decreased frequency of *IL-10* (-1082, -819,-592) ATA haplotype in patient group in comparison with control category. The scarcity of the aforementioned low-producing haplotype in our patients group could suggest IL-10 as a susceptibility factor for CHF in Iranian population. Our results are in line with a previous study performed by Bijlsma et al. (35), which detected no correlation between the aforementioned genotypes and heart failure or heart transplant rejection in patients suffering from dilated cardiomyopathy or ischemic heart failure. Karaca et al. (37) also found no associations between *IL-10* -1082 G/A and -592 C/A polymorphisms and coronary heart disease in elder subjects, although they have suggested the probable role of *IL-10* -592 C/A polymorphism in CHD susceptibility in younger patients (37). Our findings are inconsistent with the results of a very recent meta-analysis study conducted by Chao et al., which revealed the association of *IL-10* -1082 AA genotype with increased atherosclerotic

risk (38). In addition, Wang et al. (39) suggested *IL-10* -1082G/A polymorphism genotypes (GA+AA) to be associated with an increased risk of coronary heart disease, especially in Caucasians, as a result of their meta-analysis study. In another recent study, Yu et al. (40) proposed C allele with SNPs at position –592C/A and –819C/T of *IL10* gene to be associated with ischemic heart disease (IHD) in the Korean population, but observed no correlation between -1082 G/A SNPs with IHD.

In closing, we believe this is the first study in which the assessment of the associations between certain SNPs in both *IL-10* and *TGF-β1* genes and individual vulnerability to CHF has been carried out in a group of Iranian patients. Our findings unveiled great contrasts in certain genotypic positions [TGF-β1 at codon 10 (CT and CC), TGF-β1 at codon 25 (GC)], and haplotypic positions [IL-10 (-1082, -819, -592) in ATA, TGF-β1 (codon 10, codon 25) in CG and TG], between case and control groups. This association study suggests the aforementioned gene variants as possible genetic risk factors for the initiation and progression of underlying cardiovascular disorders leading to CHF. However, considering the genetic heterogeneity in studies of HF susceptibility in different races, further investigations are advocated in divergent ethnic groups, using larger sample size, to authenticate such associations between *IL-10* and *TGF-β1* gene polymorphisms and CHF.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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