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CLINICAL STUDY

Association of interleukin 1 gene cluster and interleukin 1 receptor gene polymorphisms with ischemic heart failure

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ABSTRACT

BACKGROUND: Proinflammatory cytokines have been known to play a considerable part in the pathomechanisms of chronic heart failure (CHF). Given the importance of proinflammatory cytokines in the context of the failing heart, we assessed whether the polymorphisms of interleukin (IL)-1 gene cluster, including IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA) and IL-1R gene are predictors of CHF due to ischemic heart disease.

METHODS: Forty- three patients with ischemic heart failure were recruited in this study as patients group and compared with 140 healthy unrelated control subjects. Using polymerase chain reaction with sequence-specific primers method, the allele and genotype frequency of 5 single nucleotide polymorphisms (SNPs) within the IL-1α (-889), IL-1β (-511, +3962), IL-1R (psti 1970), and IL-1RA (mspa1 11100) genes were determined.

RESULTS: The frequency of the IL-1β -511/C allele was significantly higher in the patient group compared to that in the control group (p = 0.031). The IL-1β (-511) C/C genotype was significantly overrepresented in patients compared to controls (p = 0.022).

CONCLUSIONS: Particular allele and genotype in IL-1β gene were overrepresented in patients with ischemic heart failure, possibly affecting the individual susceptibility to this disease *(Tab. 1, Ref. 27)*. Text in PDF *www.elis.sk.*

KEY WORDS: heart failure, single nucleotide polymorphism, interleukin-1.

Introduction

Chronic heart failure (CHF) is a compelling public health problem characterized by depressed contractile function and progressive ventricular dilation, with an incidence rate of 10 per 1000 population after the age of 65 (1, 2). Given the increasing economic and social impact of the disease, it stands to reason that identification of novel genetic markers, which affect individual susceptibility to the development of CHF, would be crucial for initiating the therapy at an early stage of the disease.

Elevated intracardiac and circulatory levels of proinflammatory cytokines have been hitherto revealed in patients with CHF (3–5). These cytokines' contribution towards the initiation and progression of the underlying cardiovascular diseases, especially coronary artery disease (CAD), have been a topic of intensive research recently (6, 7). Given the potential role of IL-1 in the inflammation-triggered pathway of thrombus formation, and its importance in vulnerability to the ischemic arterial disease (8), IL-1 gene polymorphisms might influence the individual proneness to ischemic heart failure (IHF).

It has been described that genetic polymorphisms within the coding and promoter regions of cytokine genes could regulate their production (9–11). Notwithstanding the fact that association of certain cytokines single nucleotide polymorphisms (SNPs) has been studied in a number of immunological diseases (12–21), our understanding in CHF is limited due to the paucity of investigations in this area. The role of polymorphisms in IL-1 gene cluster and IL-1 receptor gene in CHF has not been fully investigated and to the best of our knowledge, this is the first study examining the possible contributions of SNPs in IL-1 family genes toward susceptibility to CHF in Iranian patients.

The primary objective of this study was to determine the associations between certain IL-1 gene cluster and IL-1 receptor gene polymorphisms and CHF in a group of Iranian patients.

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Patients and methods

Subjects

Forty-three consecutive Iranian patients diagnosed with chronic ischemic heart failure (mean age 60.05 ± 11.97; 34 men, 9 women) with angiographically significant CAD, defined as ≥ 50 % diameter stenosis in at least one of the major coronary arteries, were recruited in the current study. The diagnosis of chronic heart failure was made according to the presence of impaired left ventricular systolic function (left ventricular ejection fraction ≤ 40 %) and left ventricular dilation (left ventricular end-diastolic diameter > 5.5 cm) on echocardiography. Subjects with malignancies, acute decompensated heart failure within 3 months prior to recruitment, recent myocardial infarction, and chronic lung disease were excluded. Transthoracic echocardiography and cardiac catheterization were performed for all patients. Eligible patients were in stable clinical condition and received conventional medical therapy for at least 3 months. One hundred and forty healthy individuals (mean age 45.63 ± 10.84; 101 men, 39 women), who were randomly selected from blood donors at Iranian blood transfusion organizations, were enrolled as the control group (22).

The study was approved by the Ethical Committee of Tehran University of Medical Sciences. Written informed consent was obtained from all participants before blood sampling.

Genotyping

An amount of 5 mL of peripheral blood was collected from all of the participants in this study and kept with ethylenediaminetetraacetic acid (EDTA) as anticoagulant, at –20 °C until investigation. Genomic DNA was extracted from peripheral blood leukocytes using the "salting out" technique (23). Cytokine typing was performed using polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as discussed previously (22). Amplification of the isolated DNA was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: initial denaturation at 94 °C for 2 min; denaturation at 94 °C for 10 sec; annealing + extension at 65 °C for 1 min (10 cycles); denaturation at 94 °C for 10 sec; annealing at 61 °C for 50 sec; extension at 72 °C for 30 sec (20 cycles). The presence or absence of polymerase chain reaction (PCR) products was visualized by 2 % agarose gel electrophoresis and subsequent ultraviolet transilluminator. All individuals were genotyped for 5 polymorphic sites in 4 cytokine genes, namely: IL-1α, -889 T/C; IL-1β, -511 C/T and +3962 T/C; IL-1R, psti 1970 C/T; IL-1RA, mspa1 11100 T/C.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.00 for Windows (Graphpad Software). Allele and genotype fre-

Tab. 1. Allele and genotype frequencies in patients with ischemic heart failure and healthy controls.

Cytokine	Position	Alleles/ Genotypes	Patients (n=43) n (%)	Controls (n=140) n (%)	p-value	Odds ratio (95% CI)
			n = 42	N = 136		
IL-1α	-889	C	59 (70.2)	186 (68.4)	0.789	1.09 (0.64 to 1.86)
		T	25 (29.8)	86 (31.6)	0.789	0.92 (0.54 to 1.56)
		CC	20 (47.6)	62 (45.6)	0.860	1.09 (0.54 to 2.17)
		CT	19 (45.2)	62 (45.6)	1.000	0.99 (0.49 to 1.98)
		TT	3 (7.2)	12 (8.8)	1.000	0.79 (0.21 to 2.96)
			N=42	N=139		
IL-1ß	-511	C	58 (69)	154 (55.4)	0.031	1.8 (1.07 to 3.02)
		T	26 (31)	124 (44.6)	0.031	0.56 (0.33 to 0.94)
		CC	19 (45.2)	36 (25.8)	0.022	2.37 (1.15 to 4.84)
		TC	20 (47.6)	82 (59)	0.217	0.63 (0.32 to 1.27)
		TT	3 (7.2)	21 (15.2)	0.297	0.43 (0.12 to 1.53)
			N=41	N=140		
IL-1ß	+3962	C	60 (73)	198 (70.7)	0.782	1.13 (0.65 to 1.96)
		T	22 (27)	82 (29.3)	0.782	0.89 (0.51 to 1.54)
		CC	21 (51.2)	70 (50)	1.000	1.05 (0.52 to 2.11)
		TC	18 (44)	58 (41.4)	0.858	1.11 (0.55 to 2.23)
		TT	2 (4.8)	12 (8.6)	0.739	0.55 (0.12 to 2.55)
			N=43	N=140		
IL-1R	pst-1 1970	C	58 (67.4)	174 (62.1)	0.443	1.26 (0.76 to 2.11)
		T	28 (32.6)	106 (44.2)	0.443	0.79 (0.48 to 1.32)
		CC	20 (46.5)	54 (38.6)	0.378	1.39 (0.7 to 2.76)
		TC	18 (41.9)	66 (47.1)	0.602	0.81 (0.40 to 1.61)
		TT	5 (11.6)	20 (14.3)	0.802	0.79 (0.28 to 2.25)
			N=43	N=140		
IL-1RA	mspa-1 11100	C	18 (20.9)	64 (22.9)	0.769	0.89 (0.50 to 1.61)
		T	68 (79.1)	216 (77.1)	0.769	1.12 (0.62 to 2.02)
		CC	2 (4.6)	4 (2.9)	0.627	1.66 (0.29 to 9.39)
		TC	14 (32.6)	56 (40)	0.474	0.72 (0.35 to 1.49)
		TT	27 (62.8)	80 (57.1)	0.597	1.27 (0.63 to 2.56)

quencies for all cytokine gene polymorphisms were assessed by direct gene counting. Frequencies of alleles and genotypes were compared between the case and control groups using the Fisher's exact test. The odds ratio and 95 % confidence intervals for the influence of the aforementioned SNPs on ischemic heart failure risk were calculated. A p-value less than 0.05 was regarded statistically significant.

Results

Alleles and genotype frequencies

Allelic and genotype frequencies in patients with ischemic heart failure and healthy controls are depicted in Table 1.

We found a significant positive association for IL-1β -511/C allele (69 % vs 55.4 %, p = 0.031) with ischemic heart failure. In addition, the IL-1β C/C genotype at position -511 was significantly overrepresented in patients with ischemic heart failure compared to healthy controls (45.2 % vs 25.8 %, p = 0.022).

The allele and genotype frequencies of IL-1α at position -899, IL-1β at position +3962, IL-1R at position psti1970 and IL-1RA at position mspai11100 were similar in two groups of patients and controls.

Discussion

In the present study an increased frequency of the IL-1β -511/C allele was found in patients with ischemic heart failure, whereas the T allele at the same position was significantly decreased. Moreover, the frequency of the IL-1β (-511) C/C genotype was significantly higher in our patients compared to controls. It has been shown that IL-1β (-511) C/C genotype is associated with increased in vitro IL-1β expression of mononuclear cells in response to lipopolysaccharide (LPS) (24). IL-1β -511C/T polymorphism seems to affect the risk of myocardial infarction, as the main cause of systolic heart failure, at young age (24); however, neither individual SNPs nor SNP haplotypes in the promoter region of the IL-1β gene were significantly associated with the incidence of acute coronary syndromes in patients above the age of 50 years (25). In a more recent study, IL-1β (-511) T/T genotype has been shown to be an independent predictor of left ventricular systolic dysfunction (LVSD) in patients with IHD (26). In this study, the production of IL-1β under stress conditions was dramatically less in patients with both IL-1β (-511) T/T genotype and LVSD. Hence, it has been postulated that, an inadequate response of IL-1β under stress conditions in carriers of IL-1β (-511) T/T genotype may impair the cytoprotective effects of IL-1β on the ischemic myocardium (26). Our results, on the other hand, imply that the IL-1β (-511) C/C genotype might contribute to the development of ischemic heart failure. Therefore, it could be suggested that the apparent increase in the IL-1β (-511) C/C genotype in the patient group might be responsible for the high-producer phenotype, playing prominent roles in mediating maladaptive responses in the context of the failing heart (4).

Our study has certain limitations to be acknowledged. Firstly, the relatively small number of cases in the patient group reduces the statistical power, thereby not allowing for comparison among the groups, with regards to disease severity. Therefore, given the small number of patients in this study, any conclusions can only be interpreted with caution. Furthermore, serum level of IL-1 was not measured in this investigation, which results in our inability to evaluate the relevance of the aforementioned gene variants in terms of cytokine levels in patients with ischemic heart failure.

In conclusion, this study demonstrates the association between specific allele and genotype frequencies in IL-1β gene in addition to IL-4, which was previously showed (27), with ischemic heart failure. These associations may help us define novel genetic predisposing factors in regard to this disease. However, in order to delineate the role of IL-1 family genotypes in the pathogenesis of ischemic heart failure, further investigation using a larger sample size is recommended.

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