Increased Levels of IL-23 in Peripheral Blood Mononuclear Cells of Patients With Chronic Heart Failure

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Abstract- Chronic heart failure (CHF) is a complex clinical syndrome that represents the end stage of various cardiac diseases and is characterized by the inability of the heart to meet metabolic demands of the body. Many physiological systems are involved in this disease. In particular, the activation of the immune system has received considerable interest in the last decade. Evidence from both experimental and clinical trials indicates that inflammatory mediators are of importance in the pathogenesis and progression of chronic heart failure. Excessive pro-inflammatory cytokines induce contractile dysfunction, hypertrophy, and fibrosis and cell death in Cardiac myocyte. We examined the expression of IL-23 in PBMCs between CHF patients and healthy controls. In this report, we used real-time PCR assay to compare the relative expression of IL-23 in peripheral blood mononuclear cells (PBMC) from CHF patients with various heart diseases (n=42, EF<45%, range of New York Heart Association (NYHA) 1 to 4) and matched healthy control subjects (n=42).We also determined the IL-23 concentrations of cell culture supernatant of PBMCs with ELISA. A total of 42 patients with CHF, with 42 age and sex-matched control group subjects were enrolled in the present study. The culture supernatant levels of IL-23 in PBMC of CHF patients were significantly higher (133.95±108.99 pg/mL) than in the control group (83.43±76.2 pg/mL) (P<0.05). The gene expression of IL-23 was also markedly upregulated in PBMC from CHF patients in comparison with the control group, but it was not statically significant 80. These results demonstrate that in patients with CHF and especially those with severe CHF, expression of pro-inflammatory cytokines and levels of IL-23 cytokine is markedly increased in PBMC. These finding suggested that IL-23 may play an important role in the progression of CHF among these patients.

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Keywords: Heart failure; Cytokines; Gene expression; Immunology

Introduction

Congestive heart failure (CHF) is one of the most common cardiovascular diseases, and its prevalence is increasing in developing countries (1,2). Recent evidence indicates that persistent activation of various neurohormonal pathways including inflammatory mediators play a major role in the progression of the disease (3-7). Serum levels of tumor necrosis factor (TNF) and interleukin-6 (IL-6), have been shown to be

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elevated in patients with CHF and to correlate with deteriorating functional class and predict the poorer clinical outcome (7). While several reports have shown increased circulating levels of inflammatory cytokines in CHF patients, few studies have investigated the expression of these mediators at the cellular levels (8,9). Circulating inflammatory cells may not only contribute to the systemic immune activation in CHF patients but could indirectly promote myocardial dysfunction by infiltrating the failing myocardium (10-12). Moreover, while much attention has been drawn to TNF-α and IL-6 in CHF, our knowledge of other members of the cytokine network such as IL-23 is limited. Therefore, in the present study, we aimed to examine the expression of IL-23 in peripheral blood mononuclear cells (PBMCs) between CHF patients and healthy subjects.

Materials and Methods

Forty-two patients (24 men, 18 female) with stable CHF for more than 6 months who were referred to the Tehran Heart Center from April 2014 to September 2014, were enrolled in this study. The diagnosis of heart failure was based on clinical symptoms as well as on radiological and echocardiographic findings. Clinical history, physical examination, and relevant laboratory investigations were obtained for all patients. The severity of CHF was assessed according to New York Heart Association functional class I to IV and having symptoms for at least 3 months. The exclusion criteria were as follows; coronary artery disease at angiography, diabetes, significant concomitant diseases such as infections, receiving any anti-inflammatory drugs with the exception of low dose aspirin, renal failure, autoimmune disease, and cancer. Any individuals with prior myocardial infarction (MI) were excluded because plasma cytokines may be elevated after myocardial injury (13). The control group consisted of 42 healthy subjects who were age and sex matched with no history of ischemic heart disease. This project was approved by the Ethics Committee of Tehran University of Medical Sciences. Written informed consent was gathered from the subjects prior to sampling.

Laboratory measurements of IL-6, IL-23, and TNF-α

Peripheral blood mononuclear cells (PBMC) were collected from heparinized venous blood using density-gradient centrifugation on Ficoll-Hypaque 1077 within 25 minutes. All plasma samples were stored in the supernatant at –70° C until assay. The plasma concentrations of IL-23 were assessed using commercially available ELISA assay (Ebioscience, San Diego, USA) according to the manufacturers' instructions.

RNA isolation and cDNA synthesis

Total RNA for real-time quantitative real time PCR was isolated by RNX plus Solution (Cinnagen, Iran) according to well-established protocols (14). The purity and integrity of RNA were determined by measuring the optical density ratio in 260 and 280 nm and agarose gel (1%) electrophoresis.

Real time PCR

For the quantitative analysis of mRNA expression, real time PCR was performed with a thermocycler (ABI, applied biosystems Step I plus, USA), and SYBR green Premix by Ex Taq (Takara, Japan). The amplifications containing SYBR Green 1 Dye, forward and reverse primers, and template cDNA were carried out. Melting-curve analysis was used to confirm the specificity of the amplification reaction. The results for the target genes were measured as fluorescent signal intensity and were normalized to the internal standard gene β-actin.

Statistical analysis

The data were analyzed using SPSS v.23 software. Kolmogorov Smirnov (K.S) test was used to evaluate the normal distribution of the quantitative variables. Statistical comparisons were performed using independent *t*-test (for quantitative variables) and the chi-square test (for qualitative variables). *P* less than 0.05 was considered significant in all tests. In addition, correlation analysis was used to evaluate the association between different continuous variables of the study.

Results

A total of 42 patients with CHF, with 42 age and sex-matched control group subjects were enrolled in the present study. Table 1 describes the baseline clinical characteristics of the study population. Among the CHF group, 25 patients (59.5%) were in NYHA III-IV. The etiological causes of CHF included ischemic and non-ischemic.

The culture supernatant levels of IL-23 in PBMC of CHF patients were significantly higher (133.95±108.99 pg/mL) than in the control group (83.43±76.2 pg/mL) (P<0.05, Table 2, Figure 1). Multiple comparison tests demonstrated that patients in NYHA class II (22.21±2383.3 pg/mL), as well as those in classes III and IV (108.1±54.2 pg/ mL), showed significantly higher concentrations of IL-23 in PBMC than the control subjects (P<0.01 and P<0 .001, respectively, Table 3, Figure 2).

Table 1. Demographic and clinical characteristics of CHF patients

		CHF (n=42)	Control (N=42)
Demographics	**Age, y**	55.3 ± 5.76	54.35 ± 4.8
	Male, %	57.1	61.9
	Female, %	42.9	38.1
NYHA functional class, %	**I**	14.2	0
	II	26.1	0
	III	28.5	0
	IV	30.9	0

CHF: Congestive Heart Failure, NYHA: New York Heart Association

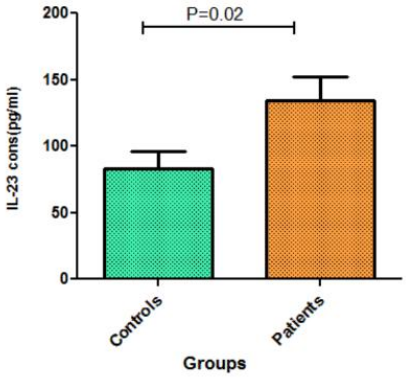

Figure 1. IL-23 level in PBMC of CHF patients and controls

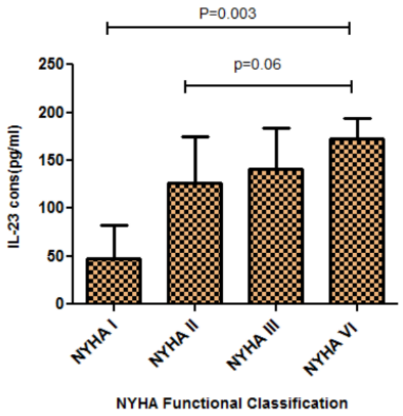

Figure 2. IL-23 level in CHF patients with different NYHA functional class

Table 2. Comparison of IL-23 level in PBMC between cases and controls

	Mean±SD	Median	Range	*P*
Cases	133.95±108.99	107.9	433.6	0.02
Controls	83.43±76.2	69.45	339.5	0.02

Table 3. Comparison of IL-23 level in PBMC of CHF patients with different NYHA class

NYHA class	Mean ± SD	Median	Range	*P*
I	47.2±77.6	11.8	183.6	0.03
II	118.2±129.4	53.3	400	0.03
III	141.5±125.8	103.7	392.4	0.03
IV	173.03±76.6	172.2	246.7	0.03

PBMC: Peripheral blood mononuclear cells

The gene expression of IL-23 was also markedly upregulated in PBMC from CHF patients in comparison with the control group, but it was not statically significant (Table 4, Figure 3) (*P*=0.1). However, no significant changes in gene expression of IL-23 were found for different NYHA classes (Table 5, Figure 4). Finally, among CHF patients, no significant differences existed between the gene expression of IL-23 between patients with ischemic and non-ischemic etiology (Table 6, Figure 5) (*P*=0.1).

Table 4. Comparison of gene expression between cases and controls

	Mean±SD	Median	Range	*P*
Cases	1.8±1.4	1.5	6.8	0.1
Controls	1.41±1.15	1.3	5.8	0.1

Table 5. The comparison of IL-23 gene expression between different NYHA classes

NYHA class	Mean±SD	Median	Range	*P*
I	1.14±0.87	0.8	2.3	0.1
II	1.33±0.78	1.14	2.54	0.1
III	2.1±1.5	1.8	5.5	0.1
IV	2.47±1.7	2.7	6.8	0.1

Table 6. Comparison of PBMC levels of IL-23 among CHF patients with ischemic or non-ischemic etiology

Groups	Mean±SD (pg/ml)	Median (pg/ml)	Range (pg/ml)	*P*
Ischemic	1 4 8.8±1 1 2.2	132.9	424.6	0.1
Non-ischemic	1 4 8.6±1 1 5.36	61.4	298.3	0.1

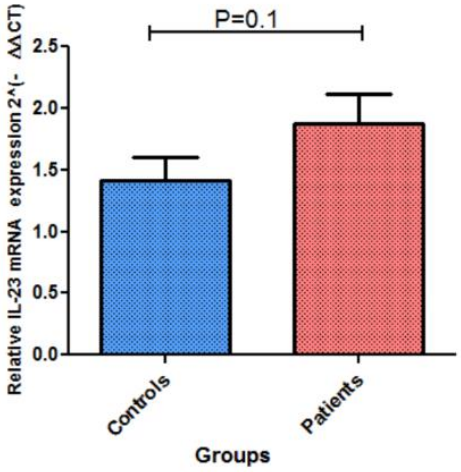

Figure 3. The gene expression of IL-23 in PBMC of CHF patients and controls

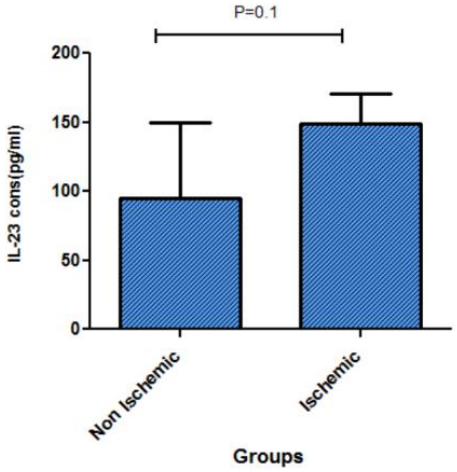

Figure 5. Gene expression of IL-23 in CHF patients with ischemic and non-ischemic etiology

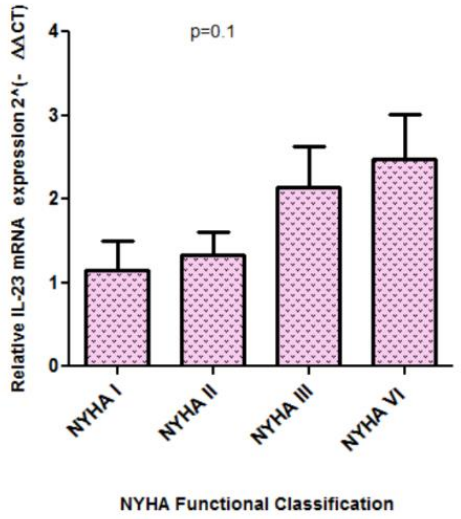

Figure 4. The gene expression of IL-23 in CHF patients with different functional class

Discussion

In this case-control study, we showed that patients with CHF have markedly enhanced levels of IL-23 in PBMC compared to controls. Also, there was increased level of gene expression among CHF patients than in control group. To elucidate the effect of the increased IL-23 levels in patients with CHF, PBMCs from patients and controls were stimulated. The results showed that gene expression of IL-23 was not significantly increased among patients in NYHA function classes I to IV.

Over the past two decades, a great deal of experimental and clinical investigations have been carried out to support a pathological role of pro-inflammatory cytokines in the development and progression of heart failure. Evidence from many studies has indicated that CHF should also be considered as a state of immune activation and not merely a failure of the heart to provide adequate cardiac output (15,16).

Although the complex nature of immune pathophysiological mechanisms in CHF is not fully understood, persistent inflammation may play a major role in the development of CHF. Previous studies have reported elevations of circulating levels of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1 in CHF patients which also correlates to the clinical severity of the disease (17). In addition, these inflammatory cytokines are believed to be responsible for the remodeling process during CHF which is characterized by hypertrophy, ventricular dilatation, and fibrosis (18,19).

The role of IL-23 in the pathogenesis of various inflammatory diseases such as atherosclerosis, (20) allergies, (21) autoimmune diseases (22,23), and allograft transplantation (24) have been previously shown. Elevated levels of IL-23 have been reported in carotid lesions among symptomatic patients and has been associated with increased mortality during follow up underscoring the association between IL-23 and carotid atherosclerosis (25).

In addition to atherosclerosis, recent reports have also implicated IL-23 as having a key role in cardiac ischemia-reperfusion injury and LV remodeling (26,27). In a mice model of myocardial infarction (MI), a functional link between IL-23 signaling axis and late-stage LV remodeling after (MI) was identified (28,29). Mice lacking IL-23 showed improved survival, limited infarct expansion, and reduced fibrosis in the non-infarcted myocardium. These findings support a direct role of IL-23 in the remodeling process of LV which can contribute to the development and progression of CHF. Our results are in line with these findings demonstrating higher levels of IL-23 in CHF patients. Therefore, it could be suggested that expression of IL-23 in PBMCs may play an important mediating role in the activation of systemic inflammation which could eventually lead to the progression of heart failure.

The present study has a number of limitations. First, the total number of patients were low which could result in the lack of association between levels of IL-23 and different NYHA classes. Second, echocardiographic evaluation was not performed at the baseline examination; therefore, limiting the ability to examine the relative contributions of subclinical LV dysfunction and inflammatory cytokines to CHF risk. Therefore the results of the present study should be interpreted in the context of its limitations.

Our investigation of the supernatant levels of IL-23 in cultured PBMC revealed that a high expression pattern of these inflammatory cytokines exists among CHF patients compared to healthy subjects. These findings suggest that elevated inflammatory markers constitute important risk factors for CHF and may provide a potential therapeutic target for preventing the development of CHF. Further studies with larger number of patients are needed.

Acknowledgments

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