Expression of Interleukin 1, Interleukin 27 and TNF α Genes in Patients with Ischemic Cardiomyopathy Versus Idiopathic Dilated Cardiomyopathy

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Abstract

Objective: Congestive Heart failure (CHF) is a complex multifactorial syndrome due to tissue hypo perfusion that is affected by some factors like inflammatory cytokines. In our study we investigated the exact gene expression of three inflammatory cytokines in ischemic and idiopathic cardiomyopathy patients.

Materials and Methods: From 49 studied recipients in ischemic group, 23 (46.9%) were male and from 40 studied recipients in idiopathic dilated cardiomyopathy group, 19 (47.5%) were male. For the quantitative analysis of Interleukin (IL)1, IL-27 and TNF-α mRNAs expression level, the SYBR Green Real-Time PCR method was performed using SYBRPremix Ex TaqTM II (Tli RNaseH Plus) (Takara, Japan) and designed primers specific for each genes in an iQ5 thermocycler (BioRad Laboratories, USA) according to the manufacturer's instructions.

Results: Our results showed that the expression level of IL-1 and TNF-α were significantly higher in the ischemic patients compared to healthy controls (P<0.001, P<0.01, respectively); also we found higher levels of IL-1 and IL-27 gene expressions in idiopathic patients compared to healthy controls (P<0.001, P<0.001, respectively). There were not any significant difference of IL-1, IL-27 and TNF-α expression levels between ischemic patients and idiopathic ones.

Conclusion: Although we would introduce IL1, IL-27 and TNF α as effective inflammatory cytokines on myocardial functions in ischemic and idiopathic cardiomyopathy patients; there is not any difference between these two groups in gene expression of three main inflammatory cytokines.

Introduction

Congestive Heart failure (CHF) is a complex multifactorial syndrome due to tissue hypo perfusion and body fluid retention that is presented by fatigue, cachexia, shortness of breath and inability to do some ordinary daily activities[1]. The most common cause of CHF is ischemic insults to myocardium (myocardial infarction) while other probable reasons such hypertension, alcohol usage, viral infection and muscle abnormalities occurred by genetic defects should not be forgotten[2]. Notwithstanding of previous studies on this chronic syndrome, there is a major question that which pathogenesis is responsible for left ventricular failure and cardiomyopathy in absence of ischemic events of myocardium[3]. About one third of CHF patients suffered from dilated cardiomyopathy (DCM). Cardiomyopathy is a condition of deterioration of myocardial function and structure that is finally leads to heart failure. In dilated ones patients suffered from dilatation of one or both ventricle size with reduction of ejection fraction. If primary and secondary etiologies excluded, dilated cardiomyopathy is considered as idiopathic (IDCM). In another word, it is defined as non-ischemic cardiomyopathy with depressed left ventricular (LV) function[4]. Cardiomyopathy also is the most common cause of heart transplantation in CHF patients[5, 6]. Not only increasing in inflammatory cytokines but also overexpression of immunological antigens is seen in cardiomyopathy patients; [3] besides that, complex impaction of inflammatory and pro-inflammatory cytokines on CHF progression not well understood [7, 8]. Two major pathways of immune activation in these patients are explained in literature: the first one is direct antigenic stimulation and the second one is immune activation secondary to cardiac injury. The last one exposes the heart to bad immune response and triggers the patients` condition [9]. Tumor Necrosis Factor alpha (TNF-α) almost is the most effective pro-inflammatory cytokine in HF patients that is not present in normal myocardium. Failed myocardium produces TNF α to blood stream but its` receptor is down regulated in inflammatory process of heart failure [10]. In animal models, systemic administration of TNF α induces dilated cardiomyopathy phenotype in mouse's myocardium [11]. Also blockade of biological effect of TNFα in humans has been shown to improve myocardial function and degree of disease severity in some surveys [12, 13].

Another cytokine that has a big role in inflammatory pathogenesis of HF is interleukin 1 (IL1) [14]. Many studies emphasize on deleterious effects of IL1 on infarction and remodeling of the heart [15]. This cytokine has an important impaction on modulating myocardial function and is increased in HF patients; [16] but according to some challengeable studies, concentrations of IL 1 in IDCM patients might not be elevated rather than control group [17]. In some other researches, an effective role of IL1 has been explained that could facilitate the immigration of macrophages and monocytes into tissues in inflammatory conditions [18]. Also administration of IL1 receptor antagonists has been revealed to be a therapeutic strategy in treating inflammatory and autoimmune conditions [19]. Another survey showed elevated levels of IL1 β mRNA in IDCM patients in a comparative study.
As a matter of fact, autoimmunity is known as a pathogenic agent in IDCM patients [21]. In another hand, raised inflammatory cytokines is not only present in IDCM but also is seen in ischemic cardiomyopathy patents [22]. Serum levels of inflammatory cytokines and their correlation with disease severity and patients’ prognosis in IDCM versus ischemic CMP patients is not still well understood. Therefor, we conducted this study in order to determine the exact expression of TNFα, IL 1 and IL 27 (as a new probable effective cytokine in inflammatory processes) genes in these two groups and will compare them to each other and control group.

**Materials And Methods**

In this case-control study we enrolled a total number of 89 non-diabetic patients, with known DCM with NYHA (New York Heart Association) class II and III who were referred to cardiology department affiliated to Namazee hospital, Shiraz, Iran between March 2016 and February 2019. The patients participated in our study were DCM ones that were diagnosed more than 3 months before the study and received optimal medical therapy according to medical guidelines and had no history of hospitalization. Of these patients, 49 were diagnosed as ischemic ones evidenced by coronary angiography consist of advanced three-vessel coronary artery disease or thrombosis-induced transmural myocardial infarction and forty patients were included in idiopathic DCM group after ruling out the possible causes by means of history, transthoracic echocardiography and coronary angiography. Also, we enrolled 49 age and sex adjusted healthy non-diabetic controls without any disease to compare with these two groups. All patients underwent two-dimensional echocardiography using a vivid E9 system (GE, Norway) and all measurements were performed according to the latest recommendations of American Society of Echocardiography [22]. The left ventricular end-diastolic volume (LVEDV) and left ventricular ejection fraction (LVEF) was calculated based on Simpson's biplane method. For homogenous sampling, the LVEDV index exceeding 100 mL/m2 and LVEF between 20-35% were considered as inclusion criteria as previously described in similar performance [15]. The present methodology met all the rules contributed to Helsinki protocol. This study was approved by the institutional review board of Shiraz University of Medical Sciences and won the approval of the Ethical Committee (IR.SUMS.MED.REC9045.39.01.93). All the experimental methods programed in the present study were in accordance with confirmed setups used in previous studies.

**Sample collection and ribonucleic acid isolation**

Five-milliliter peripheral blood was collected in Ethylenediaminetetraacetic acid (EDTA)-containing tubes from each patient at the time of diagnosis prior to chemotherapy treatment and also healthy individuals. The peripheral blood mononuclear cells (PBMCs) were isolated from each patient and controls using Ficoll-hypaque density gradient centrifugation. Total RNA was extracted by TRIZOL reagent (Invitrogen) according to the manufacturer's instructions and guidelines as previously described briefly [15, 23, 24].

**SYBR green real-time polymerase chain reaction**

For the quantitative analysis of IL 1, IL27 and TNF mRNAs expression level, the SYBR Green Real-Time PCR method was performed using SYBRPremix Ex TaqTM II (Tli RNaseH Plus) (Takara, Japan) and designed primers specific for each genes in an iQ5 thermocycler (BioRad Laboratories, USA) according to the manufacturer's instructions as previously described briefly [15,23, 24].

**Statistical analysis**

Data were analyzed by Statistical Package for Social Sciences (SPSS) software, version 18. The differences in the mean expression level of IL 1, IL 27 and TNF-α before and after chemotherapy as well as patients according to the comparison of NYHA class, LVEF and LVEDVI between patients with idiopathic and ischemic dilated cardiomyopathy which were compared independent t test. Also, the mean expression level of IL 1, IL 27 and TNF-α regarding laboratory data was analyzed by the Chi-square test.
Results

From 49 studied recipients in ischemic group, 23 (46.9%) were male and from 40 studied patients in idiopathic dilated cardiomyopathy group, 19 (47.5%) were male. The mean age of patients was 52 ± 1.6 ranged from 20–67 years and the mean age of the control group was 48 ± 3.5 ranged 19–84 years. Table 1 shows the primers and thermocycling condition for the IL-1, IL-27, TNF-α and GAPDH Transcripts.

Table 1

| Gene     | Primer sequences (5'->3')                   | PCR Product length | Thermocycling condition |
|----------|--------------------------------------------|--------------------|-------------------------|
| IL-1: F  | CTTCAGCCAATCTTCATT                         | 88bp               | 95°C/2 min, 40 cycles of 95°C/30 sec, 60°C/20 sec and 70°C/30 sec |
| IL-1: R  | CACTGTAATAAGCCATCAT                          |                    |                         |
| IL-27: F | CAGGCTCTACCCAGTAAC                         | 94 bp              | 95°C/2 min, 40 cycles of 95°C/30 sec, 57°C/20 sec and 70°C/30 sec |
| IL-27: R | AATAAAACCATCATCTCCCTAAAC                    | 80 bp              | 95°C/2 min, 40 cycles of 95°C/30 sec, 58°C/20 sec and 70°C/30 sec |
| TNF-α:F  | CAACCTCTTCTGGCTCAA                         | 94 bp              |                         |
| TNF-α:R  | TGGTGGTCTTTGTTGCTTA                        |                    |                         |
| GAPDH:F  | GGACTCATGACCACAGTCCA                       | 119 bp             | 95°C/2 min, 40 cycles of 95°C/30 sec, 57.5°C/20 sec and 70°C/30 sec |
| GAPDH:R  | CCAGTACGGGACGGGATGAT                       |                    |                         |

Table 2 displays the baseline characteristics of ischemic and IDCM patients that there were not any significant differences of demographic data of two groups. The functional status and echocardiographic findings of patients with DCM are also summarized in Table 3. We found that LVEF and LVEDVI were higher in ischemic DCM patients compared to IDCM individuals but it was not significant (p value = 0.07 and 0.11, respectively). Indeed, there was no any difference between stages of NYHA class in two groups.

Table 2

| Characteristics                  | Ischemic DCM (n = 49) | Idiopathic DCM (n = 40) | P    |
|----------------------------------|-----------------------|--------------------------|------|
| Age (y)                          | 53.21 ± 6.2           | 51.3 ± 9.5               | 0.22 |
| BMI (kg/m2)                      | 26.7 ± 3.2            | 24.3 ± 1.4               | 0.431|
| Gender (Male/n, %)               | 23 (46.9%)            | 19 (47.5%)               | 0.758|
| Risk factors                     |                       |                          |      |
| Smoking                          | 11 (22.4%)            | 8(20.0%)                 | 0.651|
| LDL-C > 130 mg/dL (n, %)         | 7(14.2%)              | 5(12.5%)                 | 0.901|
| TG > 150 mg/dL (n, %)            | 10 (20.4%)            | 7(17.5%)                 | 0.574|
| HDL-C < 40 in men or < 50 in women (n, %) | 4(8.1)              | 3 (7.5%)                 | 0.105|
| Hypertension                     | 9 (18.3%)             | 8 (20.0%)                | 0.13 |
Table 3
The comparison of NYHA class, LVEF and LVEDVI between patients with idiopathic and ischemic dilated cardiomyopathy

| Variable   | Idiopathic DCM, n = 49 | Ischemic DCM, n = 40 | P value |
|------------|------------------------|----------------------|---------|
| NYHA class (n, %) | 16 (32.6%) | 16 (40.0%) | 0.50 |
| LVEF (%) | 26.8 ± 4.07 | 31.57 ± 6.21 | 0.07 |
| LVEDVI (mL/m2) | 143.29 ± 29.1 | 135.25 ± 39.4 | 0.11 |

Data was expressed as mean ± SD or numbers. DCM: dilated cardiomyopathy, NYHA: New York Heart Association, LVEF: left ventricular ejection fraction, LVEDVI: left ventricular end-diastolic volume index.

IL-1, IL-27 and TNF-α gene expression in Ischemic patients and controls

The mRNA expression analysis of IL-1, IL-27 and TNF-α gene measured as Ct (cycle threshold) and ΔCt values. Our results showed that the expression level of IL-1 and TNF-α were significantly higher in the ischemic patients compared to healthy controls (P < 0.001, P < 0.01, respectively). However, no significant difference was observed between IL-27 in the Ischemic patients compared to healthy controls.

IL-1, IL-27 and TNF-α gene expression in Idiopathic patients and controls

After the statistical analysis, our results revealed that the expression level of IL-1 and IL-27 were significantly higher in the idiopathic patients compared to healthy controls (P < 0.001, P < 0.001, respectively). However, no significant difference was observed between TNF-α in the idiopathic patients compared to healthy controls.

IL-1, IL-27 and TNF-α gene expression in Ischemic patients and Idiopathic (Left) patients

Our results demonstrated that the expression level of IL-1, IL-27 and TNF-α were not significantly higher in the idiopathic patients compared to ischemic ones.

Discussion

In our study we investigated the exact gene expression of three inflammatory cytokine in CHF patients. So, we found that IL-1 and TNFα were higher in ischemic DCM patients than controls and gene expression of IL-1 and IL-27 also were higher in IDCM than control group; while there was no any significant differences of TNF α between these two groups.

TNFα and IL-1 are two cytokines with negative inotropic effects on the myocardium by uncoupling β-adrenergic signaling; [25] they have a big role on myocardial remodeling and hypertrophy after myocardial infarction [26]. Notwithstanding many previous researches on CHF, this fact that myocardial insults provoke inflammatory cytokines activity or inflammatory system deteriorate the condition of patients by activating direct antigenic pathways and immune system, is remained as a puzzle [9]. Van Tassell and his colleagues in an experimental study showed that overexpression of IL-1 gene in systolic heart failure patients can lead to poor exercise tolerance. They did their study to discover any new approach to treatment of heart failure patients and finally proposed IL-1β blockade as a novel treatment for future success approaches in treatment of these patients [27]. Also in another study, anakinra as a IL-1 blockade agent was used to reduce systemic inflammatory response in acute decompensated HF patients and finally their results supported their hypothesis [28]. In consistent with these, Kang YM et al. observed significant increasing of brain pro-inflammatory cytokines (IL-1) in ischemic induced heart failure rats [29]. In another hand, researchers examined whether the blockade of TNF α as a new neurohormonal pathway could be effective in treatment of HF patients or not. Finally they found out that this blockade is a well-established way to improve functional status and myocardial functions [12].
Interleukin 27 as a novel member of IL-12 group cytokines is high in patients with coronary artery disease [30, 31] but it is significantly higher in IDCM patients than control group while this correlation was not significant between ischemic DCM ones and controls.

It is believed that myocardial ischemic insults enforce myocardium to produce TNF α and inflammatory process is known to has a significant correlation with coronary atherosclerotic events in CHF patients. In a big database trial, Vesnarinone Trial (VEST), Deswal et al. concluded that the most important inflammatory cytokines such as TNFα and interleukin 6 have more gene expression in ischemic DCM than idiopathic DCM patients [33]. Another study that supports lower expression of TNF-α and IL-1 genes in IDCM patients is Bironaite study [17]. But we did not find any significant differences of any cytokine between ischemic DCM patients and IDCM ones. In a similar study that was carried out by Iravani Saadi et al. they compared IL-6 and IL-18 as two effective inflammatory cytokines between ischemic DCM and IDCM. They found that these two cytokine have higher expression in IDCM and ischemic ones than control group [15] but in the same line of our study, they did not find any significant difference between IDCM and ischemic DCM patients compared to each other. According to these findings we can produce IL-27 as a novel inflammatory cytokine in HF patients particularly in ischemic DCM ones. Moreover, it is hopeful to describe new pathways of therapy guidelines in HF patients as a complex syndrome with complex treatments.

Limitations

HF patients due to ischemic insults after myocardial infarctions were more frequent than idiopathic ones. Our small sample size due to exclusion of decompensated patients was one of our shortages and limitations of this study. Also, we suggest enrolment of all stages of heart failure patients and evaluation of any stages correlations with expression of different cytokines for future studies.

Conclusion

In our study we found that there were not any significant differences of inflammatory cytokines (TNFα, IL-1 and IL-27) between ischemic DCM and IDCM patients. IL-27 as a newly introduced cytokine was higher in IDCM patients than controls. TNFα was not significantly higher in IDCM than controls. Additionally, IL-27 did not have higher expression in ischemic DCM patients than controls but some surveys have been introduced it as an effective factor on myocardial functions in ischemic/reperfusion events. According to these antitheses in our results and other researches and importance of the matter, further studies are recommended in future.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences and all patients signed a predefined written inform consent before entering the trial.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are not publicly available due internal ethical policies (sample bank of cardiomyopathy patients available in "Muhammad Rasoololah Research Tower affiliated with Shiraz University Of Medical Sciences") and privacy maintenance of patients’ data but are available from the corresponding author on reasonable request.
Consent for publication
Not applicable

Competing interests
All authors declare no competing financial interests exist.

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Authors' contributions
MIS, JS, HA, EA, MAB & AM set up the study design and interpreted the data. AM, ZK & AN performed the statistical analyses, interpreted the data and drafted the manuscript. AM, HA & AM revised the manuscript critically and provided continuous guidance throughout the study. All authors read and approved the final manuscript.

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