INCREASED EXPRESSION OF CARDIOTROPHIN-1 IN CARDIOMYOPATHY PATIENTS

Sharif S1,*, Saleem A1, Naz S1, Rashid F1, Iqtedar M2, Kaleem A2, Latif A1

*Corresponding Author: Dr. Saima Sharif, Department of Zoology, Lahore College for Women University, Jail Road, Lahore, Pakistan. Tel: +92-333-409-2232. Fax: +92-042-9920-3077. E-mail: saimasharif04@gmail.com

ABSTRACT

Cardiomyopathy (CM) is a condition of cardiac dysfunction. It is one of the leading causes of mortality in which both genetic and environmental factors are involved. Cardiotrophin-1 (CT-1) level in plasma is associated with CM. It affects the cardiomyocyte differentiation. To evaluate the expression of CT-1 in cardiomyopathy, this study was done on CM subjects attending the Fatima Memorial Hospital, Lahore, Pakistan, between January and June, 2016. A total of 40 subjects were enrolled who were divided into two groups; CM group (n = 20) and a control group (n = 20). A self-designed questionnaire was filled in by each subject to collect data regarding age, body mass index (BMI) and CM history. RNA was isolated from blood after its quantification, cDNA was prepared and reverse-transcriptase-polymerase chain reaction (RT-PCR) was performed for expression of CT-1. The mean age in CM subjects was 40.1 ± 6.03 years, while it was 35.0 ± 3.7 years in the control group. The mean expression of CT-1 in the CM subjects was 5.2 ± 0.66, while it was 1.00 ± 0.001 in the control group. A highly significant difference was observed in CT-1 expression in the CM group, and expression was significantly correlated with age and BMI in CM subjects.

Keywords: Cardiomyopathy (CM); Cardiotrophin-1 (CT-1); Gene expression; Pakistani population; Reverse-transcriptase-polymerase chain reaction RT-PCR.

INTRODUCTION

Cardiomyopathy (CM) is a progressive disease of the myocardium or heart muscle, resulting in heart failure [1]. Heart muscle disorders occur due to a heterogeneous group of CM. In the absence of abnormal loading conditions or ischemic heart disease, abnormal myocardial structure and function is present in CM [2]. In the autosomal dominant forms of CM incomplete expression is common. On the basis of morphology and function, CM is classified into four groups: dilated CM (DCM), hypertrophic CM (HCM), restrictive CM (RCM) and arrhythmogenic right ventricular (RV) CM/dysplasia (ARVC/D) [2]. Worldwide, the most widespread CM is DCM. Dilated CM is a disorder in heart muscles, in which left or both ventricles become dilated and perform poor function [3]. More than 1400 mutations are associated with CM. Most of these mutations are located on genes encoding the proteins of thick and thin sarcomere filaments. Small numbers of mutations have been observed in genes which encode Z-disc components and handle calcium proteins [4]. The most common causes of CM are viral infection, alcohol, family history, age, sex, hyperglycemia, diabetes mellitus, abnormal thyroid function and heart attack. Symptoms of heart failure (HF) may include shortness of breath, fatigue, cough, orthopnea, paroxysmal nocturnal dyspnea, and edema [5,6]. Some physical activities (vigorous, moderate and sedentary life style) and etiological attributes may contribute in this disease [7].

Cardiotrophin-1 (CT-1) is an interleukin-6 (IL-6) family cytokine and is an active inducer capable of cardiac hypertrophy and vascular stiffness in hypertensive heart disease [8]. It is capable of recapitulating the physiological growth of the heart including transient and reversible hypertrophy of the myocardium [9]. In the human aortic vascular smooth muscle cells, CT-1 stimulate the proliferation,
migration and collagen-1 (COL1) expression. In vascular endothelial cells and monocyte migration, proatherogenic expression is stimulated by CT-1. Atherosclerotic lesions formed by formation of foam cells and COL1 production [10]. The purpose of present study was to examine the expression of CT-1 in CM in the local population.

RESULTS

The demographic characteristics are presented in Table 1. In the control group, 80.0% were males and 20.0% were females. In the CM group, 45.0% were males and 55.0% were females. Systolic blood pressure (SBP) and diastolic BP (DBP) of the CM group was 124.0±0.7 and 83.0±0.8 mmHg, respectively, as compared to the control group in which SBP was 103.0±0.9 and DBP was 79.0±1.5 mmHg, respectively. Etiological attributes of the disease were idiopathic 65.0%, nutritional 15.0% and multifactorial 15.0%. In the CM group, 15.0% were former smokers but in control group no one was a smoker. Symptomatology of the disease includes 85.0% breathlessness, palpitation 75.0% and chest pain was observed in 40.0% of the subjects. On the basis of physical activity, three categories were formed: vigorous activity, moderate activity and sedentary lifestyle. In control group, 20.0% showed vigorous activity, 10.0% sedentary lifestyle and 70.0% were moderately active. In DCM group, 5.0% showed vigorous activity, 20.0% showed moderate activity and 75.0% had sedentary lifestyle (Table 2).

The expression of CT-1 in the CM group was in the range of 3.8 to 8.6 with the mean value of 5.2±0.66, while for the control group, gene expression level was 1.00. Consequently, the CT-1 gene expression level was significantly increased (p <0.05) in the CM group as compared to the control group (Figure 1).

Pearson correlation coefficient between CT-1 and other parameters revealed a highly significant relationship with age and BMI (Figure 2), while non significant correlation with SBP and DBP (Table 3). In the control group, the value of expression is one so correlation cannot be calculated.

Table 1. Demographic parameters of the cardiomyopathy and control groups.

| Parameters     | CM Group (n=20) | Control Group (n=20) |
|----------------|-----------------|----------------------|
| Age (years)    | 40.10±6.03      | 35.00±3.71           |
| BMI (kg/m²)    | 25.11±0.39      | 24.22±0.66           |
| SBP (mmHg)     | 124.0±0.7       | 103.0±0.9            |
| DBP (mmHg)     | 83.0±0.8        | 79.0±1.5             |
| CT-1 (arbitrary units) | 5.2±0.34 | 1.00 |

CM: cardiomyopathy; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; CT-1: cardiotrophin-1 gene.
DISCUSSION

Among cardiovascular problems, CM is one of the leading causes of heart transplantation. Dilated CM occupies a significant position among all types of CM. In CM, more than 1400 mutations are linked [4]. Approximately 80.0% of identified mutations relating to cardiac β-myosin heavy chain and cardiac myosin binding protein C are present in eight sarcomere genes in CM [11]. Left ventricular dilation and dysfunction characterized by primary myocardial disease is known as DCM. Ventricular hypertrophy is increased mainly due to volume overload [12]. Prevalence of CM in the USA is as low as 0.02 to 0.2% in the population. It is found to be only 0.5% within unselected patients referred for echocardiography examination. Occurrence of one form of CM in Japan is the same as in the western population, namely 17.3/100,000 [13]. In Pakistan, data regarding the occurrence of CM has hardly been reported.

In comparison to the control group, the alteration in “CT-1 gene expression” in the blood cells of cardiomyopathic subjects was observed at the molecular level. In our study, RT-PCR analysis revealed 5.2-times more expression and upregulation of the CT-1 gene in the CM group than controls. Another study by Jougasaki et al. [14], confirmed the overexpression of cardiotrophin level in CM patients, in which levels of cardiotrophin mRNA in failing LV cardiotrophin from the DCM patients was assessed by semi-quantitative RT-PCR. It demonstrated the CT-1 gene expression by northern blot analysis and found that the level of gene was high in HF models. Strong positive correlation exists between left ventricular mass index and CT-1 mRNA. In congestive HF (CHF) models immunoreactivity of CT-1 was more intense in atrium and ventricle of model heart as compare to normal heart [14].

The studies revealed that direct relationship exist between increased expression of CT-1 gene and dilation of the LV. Cardiotrophin-1 acts as a marker in the progression of ventricular hypertrophy. It is obvious that early developmental genes are related to the onset of CM. Additionally, researchers recently used human myocardial tissue and found changes in the expression of these genes in heart disease patients. Our results for the expression of
the CT-1 gene in CM patients compared to healthy individuals are similar to the study by Freed et al. [15]. Our data indicated that adipose tissues are identified as a CT-1 source in CM subjects, as significant direct association between BMI and CT-1 expression was observed. High levels of CT-1 in metabolic syndrome was also reported by Natal et al. [16]. Circulating stem cell progenitor cells expressed early cardiovascular genes in peripheral blood system that resides in the bone marrow. This indicates that the peripheral blood system can be used as a marker to detect the gene expression in response to a disease. Our results supported this hypothesis that gene expression in blood cells may be the reflection of the disease harshness as high levels of plasma CT-1 were found in patients with CM. Furthermore, in another study by Tsutamoto et al. [17], the association between plasma level CT-1 and the mass index of the LV and neurohumoral factors such as norepinephrine and angiotensin II, which can stimulate in vitro production of CT-1 in CM subjects, was reported. Thus, it was concluded that expression of CT-1 gene is increased in CM patients. Increased expression of gene alters the activity of myocytes that result in the proliferation of cells and increase ventricular mass, resulting in cardiac failure.

**Conclusions.** It was concluded that expression of the CT-1 gene was significantly greater in CM subjects when compared to control subjects. Moreover, age and BMI also influence the expression.

**Acknowledgments.** We are sincerely grateful to the subjects for their help in the clinical evaluation and data collection. I am also grateful to the Pakistan Science Foundation for funding the presentation of this article at the 5th International Conference on Prehypertension, Hypertension and Cardiometabolic Syndrome, held at Venice, Italy, February 22-25 2018.

**Declaration of Interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**REFERENCES**

1. Colucci WS, Braunwald E. Heart failure: Cardiac function and dysfunction. In: Colucci WS, Editor. Atlas of Heart Diseases, 4th ed. Philadelphia, PA, USA: Blackwell Science. 1995: 128-142.
2. Maron BJ, Towbi, JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: An American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006; 113(14): 1807-1816.
3. Rosamond W, Flegal K, Furie K, Go A, Greenland K, Haase N, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2008 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008; 117(4): e25-e146.
4. Hershberger RE, Lindenfeld J, Mestroni, L, Seidman, CE, Taylor MR, Towbin JA; Heart Failure Society of America. Genetic evaluation of cardiomyopathy--A Heart Failure Society of America practice guideline. J Card Fail 2009; 15(2): 83-97.
5. Weigner M, Morgan JP. The causes of dilated cardiomyopathy. UpToDate. 2007; 15(3): 1-10.
6. Grenier MA, Osganian SK, Cox GF, Towbin JA, Colan SD, Lurie PR, et al. Design and implementation of the North American pediatric cardiomyopathy registry. Am Heart J. 2000; 139(2 Pt 3): s86-s95.
7. Watanabe T, Konii H, Sato K. Emerging roles of cardiotrophin-1 in the pathogenesis and biomarker of atherosclerosis. J. 2018; 1(1): 94-105.
8. Abdul-Ghani M, Suen C, Jiang B, Deng Y, Wedrick JJ, Putinski C, et al. Cardiotrophin 1 stimulates beneficial myogenic and vascular remodeling of the heart. Cell Res. 2017; 27(10): 1195-1215.
9. Konii H, Sato K, Kikuchi S, Okiyama H, Watanabe R, Hasegawa A, et al. Stimulatory effects of cardiotrophin-1 on atherosclerosis. Hypertension. 2013; 62(5): 942-950.
10. Chomczynski P, Mackey K. Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. Biotechniques. 1995; 19(6): 942-945.
11. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiomyopathy: A cardiac myosin heavy chain gene missense mutation. Cell. 1999; 62(5): 999-1006.
12. Thomas DE, Wheeler R, YousufZR, Masami ND. The role of echocardiography in guiding management in dilated cardiomyopathy. Eur J Echocardiogr. 2009; 10(8): 15-21.
13. Miura K, Nakagawa H, Morikawa Y, Sasayama S, Matsumori A, Hasegawa K, et al. Epidemiology of idiopathic cardiomyopathy in Japan: Results from a nationwide survey. Heart. 2002; 87(2): 126-130.
14. Jougasaki M, Tachibana I, Luchner A, Leskinen H, Redfield MM, Burnett JC Jr. Augmented cardiac cardio-trophin-1 in experimental congestive heart failure. Circulation. 2000; 101(1): 14-17.

15. Freed DH, Cunnington RH, Dangerfield AL, Sutton JS, Dixon IMC. Emerging evidence for the role of cardiotrophin-1 in cardiac repair in the infarcted heart. Cardiovasc Res. 2005; 65(4): 782-792.

16. Natal C, Antonia Fortuño M, Restituto P, Bazán A, Colina I, Diez J, et al. Cardiotrophin-1 is expressed in adipose tissue and upregulated in the metabolic syndrome. Am J Physiol Endocrinol Metab. 2008; 294(1): 52-60.

17. Tsutamoto T, Wada A, Maeda K, Mabuchi N, Hayashi M, Tsutsui T, et al. Relationship between plasma level of cardiotrophin-1 and left ventricular mass index in patients with dilated cardiomyopathy. J Am Coll Cardiol. 2001; 38(5): 1485-90.
