

## INCREASED EXPRESSION OF CARDIOTROPHIN-1 IN CARDIOMYOPATHY PATIENTS

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### 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. Cardiotoxin-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  $40.1 \pm 6.03$  years, while it was  $35.0 \pm 3.7$  years in the control group. The mean expression of CT-1 in the CM subjects was  $5.2 \pm 0.66$ , while it was  $1.00 \pm 0.001$  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); Cardiotoxin-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].

Cardiotoxin-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,

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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.

**METHODOLOGY**

The cross-sectional study was conducted at the Fatima Memorial Hospital (FMH) of Lahore, Pakistan. Permission for sampling was obtained from the Ethics Committee of the institution. Samples were collected from the Cardiology Department of FMH between January and June 2016. The sample size was calculated using sample calculator on Raosoft Inc. ([http://www.raosoft.com/sample\\_size.html](http://www.raosoft.com/sample_size.html)) with 5.0% margin of error and 50.0% confidence interval with expected prevalence of cardiac diseases as 410/ 100,000 in our population.

The study population was divided into two groups: the control group consisted of 20 healthy individuals and the CM group included 20 subjects. Inclusion and exclusion criteria were made for appropriate selection of patients which were as follows: patients with suspected HF, patients with left ventricular (LV) dilatation and dysfunction were included in the study. Other acquired or congenital cardiac diseases such as myocardial infarction, other coronary vascular disease, myocarditis, pericardial diseases (not mild pericardial effusion that may be secondary to HF) and patients of any cardiac/genetic disease were excluded from the study. Written informed consent was obtained from the subjects before their participation in the study. A structured questionnaire used for data collection regarding age, gender, habits, duration of disease and family history of disease. A blood sample (3 mL) was drawn from the enrolled subjects and transferred immediately to EDTA-containing vacutainers and mixed gently for 1 min. to prevent blood clotting and inhibiting activity of nucleases. The sequence of human gene *CT-1* was retrieved from the National Center for Biotechnology Information (NCBI) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and the BLAST (basic local alignment tool) in the bioinformatics tool ([www.genome.ucse.edu](http://www.genome.ucse.edu)). Only exomic sequences (NM-001330.5) were used for designing the primers ([www.genome.ucse.edu](http://www.genome.ucse.edu)).

RNA was isolated by the trizole method [10]. RNA quantity and quality was determined using the Nano Drop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Then, cDNA synthesis was done by the reverse transcriptase method. Furthermore, cellular gene expression was determined by reverse-transcriptase-polymerase chain reaction (RT-PCR). Statistical analysis was done using the Statistical Package for the Social

Sciences (SPSS), version 23 (IBM Corporation, Armonk, NY, USA). The statistical difference between the groups was analyzed by the Student’s *t*-test. Pearson correlation was done to determine the relationship between the *CT-1* gene and demographic characteristics.

**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 life style. 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/m <sup>2</sup> )	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
<i>CT-1</i> (arbitrary units)	5.29±0.34	1.00

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

**Table 2.** Prevalence of physical activity, etiological attributes and symptomatology in the cardiomyopathy group.

Variables	CM Group (n=40)
Physical activity (%)	
vigorous	5.0
moderate	20.0
sedentary	75.0
Etiological attributes (%)	
idiopathic	65.0
nutritional	15.0
multifactorial	15.0
Symptomatology (%)	
breathless	85.0
palpitations	75.0
chest pain	45.0

**Table 3.** Correlation between the *cardiotrophin-1* gene and demographic parameters in cardiomyopathy group.

Parameters	r Values	p Values
Age (years)	0.54 <sup>a</sup>	<0.05
BMI (kg/m <sup>2</sup> )	0.46 <sup>b</sup>	<0.01
SBP (mmHg)	-0.079	>0.05
DBP (mmHg)	0.069	>0.05

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

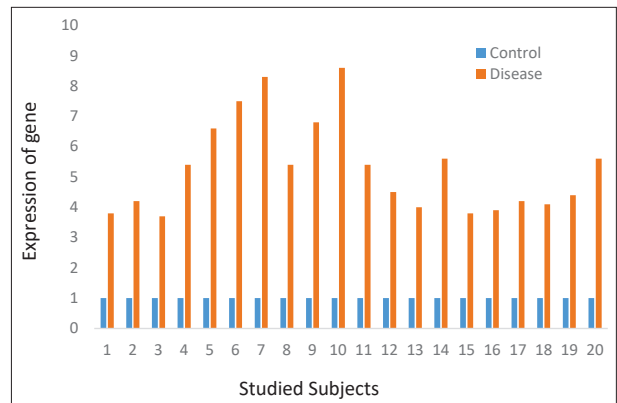
<sup>a</sup> Significant difference.

<sup>b</sup> Highly significant difference.

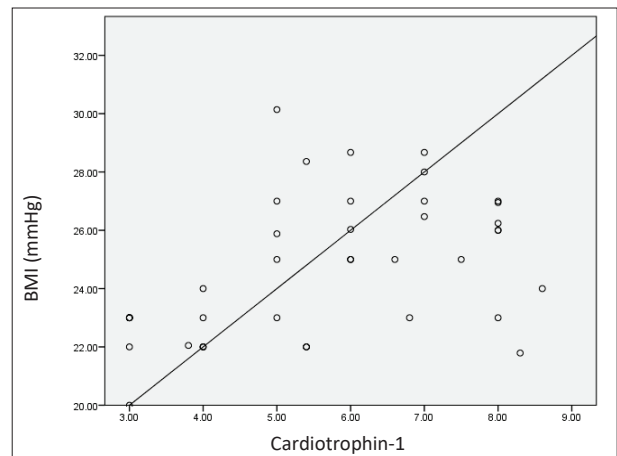
## DISCUSSION

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  $\beta$ -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



**Figure 1.** *Cardiotrophin-1* gene expression in the control and CM groups.



**Figure 2.** Scattered plot showing correlation between *CT-1* gene and BMI in CM group.

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.

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

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