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Comparison of Calcitonin Gene Related Peptide Level between Children with Dilated Cardiomyopathy and Control Group

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ABSTRACT

Background: Dilated cardiomyopathy is revealed with left ventricular dilatation and systolic dysfunction.

Objectives: This study aimed to compare the children with dilated cardiomyopathy and control group regarding the level of Calcitonin Gene Related Peptide (CGRP) and its relationship with echocardiography findings

Patients and Methods: This case-control study was conducted on 37 children with dilated cardiomyopathy and free of any clinical symptoms and 37 healthy age- and sex-matched children referring to Ali-e-Asghar and Ali Ebne Abitaleb hospitals in Zahedan, Iran. After taking history, echocardiography was performed for both groups. The data were analyzed using the SPSS statistical software and appropriate statistical tests.

Results: The two groups were significantly different regarding most of the echocardiographic parameters ($P < 0.05$). Also, a significant difference was found between the two groups concerning the mean CGRP levels ($P = 0.001$). Among echocardiographic parameters, CGRP was directly related to Interventricular Septal dimension in Systole (IVSS) ($P = 0.022$, $R = 0.375$). However, no significant relationship was observed between CGRP level and Ross classification.

Conclusions: The findings of this study showed an increase in CGRP serum levels in the case group. Besides, a direct correlation was observed between CGRP level and IVSS.

►*Implication for health policy/practice/research/medical education:*
The results of the present study have diagnostic application in early state and could be useful in clinics and researches.

1. Background

Dilated cardiomyopathy is revealed with left ventricular dilatation and systolic dysfunction. Diastolic dysfunction and damage to the right ventricle can occur, as well. In this case, patients can be at risk of right, left, or both ventricular failures. In addition to heart failure, life-threatening risk factors, such as ventricular arrhythmia, atrioventricular block, syncope, and sudden death, can also take place (1, 2).

Dilated cardiomyopathy is the most common type of cardiomyopathy (5 in 100000 for adults and 0.57 in 100000 for children) and the third cause of heart failure in Americans after coronary artery disease and hypertension. Hypertrophic cardiomyopathy is the main cause of sudden death in Athletes (1 in 500) and it is autosomal dominant. However, restrictive and arrhythmogenic cardiomyopathies of the right ventricle are rare (3).

According to a report, lymphocytic myocarditis and left ventricular non-common cardiomyopathy were two major cardiomyopathies in childhood in Australia (4).

Calcitonin neurotransmitter peptide 37 is an amino acid derived from calcitonin gene and widely spread in nervous and cardiovascular systems. Calcitonin has positive inotrope and chronotrope effects on the heart and is the strongest endogen vasodilator peptide that has been known so far (5, 6). Calcitonin Gene Related Peptide (CGRP) has positive

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inotropic and negative lusitropic effects on the heart, which is agreement with sarcomere, cardiomyocyte, and left ventricle in vivo and in vitro. The effects of CGRP are enhanced by estrogen and progestron and are partly mediated by the PK3K/AKt signaling pathway (7). To date, contradictory reports are available about calcitonin levels in Congestive Heart Failure (CHF) and only a little information is there regarding regulation of CGRP function (5, 6, 8).

Cardiac sensory nerves play a protective role in the heart through expulsion of nitric oxide and CGRP. CGRP plays a defending role in myocardial injuries, including ischemia recirculation and cardiotoxic agent doxorubicin. CGRP is not only a vasodilator with great power, but it also has beneficial effects on the heart by local down regulation of tumor necrosis factor-alpha and up regulation of insulin-like growth factor (9).

Dvorakova conducted a study on rats with long-term diabetes and demonstrated that CGRP constituted a potential therapeutic pharmacological target for treatment of cardiomyopathy (10). Moreover, the previous studies indicated that in children with Congenital Heart Disease (CHD) with secondary pulmonary volume overload, variation in CGRP level was related to the pulmonary volume overload (5). CHD with pulmonary volume overload is a cause of CHF and increase of CGRP is associated with severity of the disease (5). In most infants and children, the most common cause of CHF is CHD with pulmonary volume overload. It also plays an important role in CHF pathogenesis secondary to CHD. Besides, a few studies have suggested that pulmonary volume overload may have an important role in regulation of CGRP in children with CHF (5, 8).

In patients with hemodialysis, CGRP level is related to the volume overload. A previous study demonstrated that calcitonin had inotrope and chronotrope effects on the heart and was a central and peripheral vasodilator (6, 8). Up to now, no studies have been conducted on the relationship between CGRP level and dilated cardiomyopathy in Iran and few researches in this area are available in the world.

2. Objectives

The present study aims to compare the children with dilated cardiomyopathy and the control group regarding CGRP level to determine its relationship with echocardiographic findings.

3. Patients and Methods

This case-control study was conducted on 37 patients and 37 healthy children in Ali-e-Asghar and Ali Ebne Abitaleb hospitals in Zahedan, Iran in 2012 - 2013. The evaluated patients aged from 1 to 18 years. The exclusion criteria of the study for the case group were hemoglobin < 10 gr/dL and suffering from endocrine and metabolic disorders, valvulopathy, dysrhythmia, or blocks. The control group participants had no diseases and had referred to the hospital for routine check-up.

Taking history, physical examination, CXRay, ECG, and echocardiography by My lab 60 with transducer 3, 8 (made in Italy) were performed for all the participants. After diagnosis, they were divided into two groups. Prior to the study, the patients' weight, height, and hemoglobin were evaluated. Height was measured using a scaled table in sleeping position for the children below 2 years old and by a scale in standing position for others. Besides, weight was calculated using a special Mika scale (made in Japan) for infants and by Rasa scale (made in Iran) for other children. In addition, Ross modified classification was used to evaluate heart failure. Finally, 3 ml blood were taken from all the cases and controls and after removing the plasma, all the samples were kept at -80 ºC and were then sent to laboratory for determining the CGRP levels using an ELISA kit. After that, they were compared to the echocardiographic findings.

After all, the study data were entered into the SPSS statistical software, version 20 and were analyzed using descriptive statistics and independent t-test. In case the data were not normally distributed, non-parametric methods, such as Mann-Whitney test, were used. The limitations of this study were low prevalence of the disease, long study period, and high cost of the utilized kits.

4. Results

This study was conducted on 74 children, including 37 patients with cardiomyopathy (50%) and 37 healthy children (50%). The youngest subject was 1 year old and the oldest one was 18 years old. The mean age of the children was 12.16 ± 4.56 years in the case group and 10.56 ± 4.56 years in the control group, but the difference was not statistically significant (P = 0.348). In addition, the children's mean weight was 35.1 ± 18.4 and 28.7 ± 10.5 kg in the control and case groups, respectively and the difference was not statistically significant (P = 0.097). Finally, the children's mean of height was 128.7 ± 29.1 cm in the control group and 127.4 ± 18.1 cm in the case group, but the difference was not statistically significant (P = 0.834). However, the means of LVDD, LVESD, MPI, ICT, IRT, PEP/ET, IVSDD, LVPWDD, IVSDS, and LVPWDS were lower and those of peak E velocity and ET were higher in the control group compared to the case group (P < 0.05). The mean levels of CGRP were 2.27 ± 1.58 and 1.61 ± 0.54 pg in the case and control groups, respectively and the difference was statistically significant (P < 0.001). Nonetheless, no significant difference was observed between the two groups concerning some parameters, such as peak A velocity, E/A ratio, LA/Ao, PEP, EF, and FS (P > 0.05).

The results of Pearson's correlation coefficient (r = 0.375) revealed a significant correlation between Interventricular Septal dimension in Systole (IVSS) and CGRP in the case group (P = 0.022) (Table 1).

According to Table 2, the means of all the parameters, except for ET, were lower in the control group than in the case group. Besides, a significant difference was observed between the two groups regarding the means of MPI, IRT, DT, peak E velocity, peak A velocity, PEP, and PEP/ET (P < 0.05).

According to Figure 1, the CGRP levels 1 - 2 had the highest frequency in the study population, with the frequency being higher among the controls compared to the cases. By increasing the level of CGRP, the frequency decreased down to levels 3 - 4. No control subjects were there in levels above 4.

The correlation between CGRP and echocardiographic parameters in left and right hearts has been presented in

Table 1. Mean and Standard Deviation of Echocardiographic Parameters in the Left Heart and CGRP

Parameter	Case Mean ± SD	Control Mean ± SD	P value
Ventricular end-diastolic volume	66.10 ± 22.12	39.99 ± 14.78	0.000
Ventricular end-diastolic dimension	46.58 ± 7.71	39.993 ± 4.09	0.000
Ventricular end-systolic dimension	30.18 ± 7.45	26.51 ± 3.22	0.009
Ejection Fraction	62.51 ± 13.71	63.53 ± 5.50	0.681
Fractional shortening	35.40 ± 8.06	33.33 ± 3.73	0.171
Myocardial performance index	0.54 ± 0.18	0.32 ± 0.051	0.000
Isovolumic contraction time	0.02 ± 0.009	0.01 ± 0.007	0.000
Isovolumic relaxation time	0.10 ± 0.02	0.09 ± 0.01	0.018
Acceleration time	0.05 ± 0.01	0.05 ± 0.008	0.976
Deceleration time	0.12 ± 0.01	0.12 ± 0.01	0.572
Peak E velocity	85.9 ± 17.75	101.5 ± 19.64	0.001
Peak A velocity	55.37 ± 15.50	57.54 ± 15.81	0.574
E/A velocity ratio	1.65 ± 0.75	1.84 ± 0.46	0.245
Atrium aortic ratio	1.25 ± 0.29	1.20 ± 0.15	0.386
Pre-ejection period	0.12 ± 0.15	0.09 ± 0.10	0.314
Pre-ejection period/ejection time	0.35 ± 0.05	0.29 ± 0.03	0.000
Ejection time	0.25 ± 0.02	0.26 ± 0.01	0.024
Interventricular septal dimension in diastole	6.31 ± 1.29	5.43 ± 0.88	0.002
Ventricular posterior wall dimension in diastole	4.21 ± 1.22	3.34 ± 0.50	0.000
Interventricular septal dimension in systole	9.27 ± 1.76	8.28 ± 1.08	0.006
Ventricular posterior wall dimension in systole	4.25 ± 1.24	3.36 ± 0.52	0.000
Calcitonin gene related peptid	2.81 ± 1.92	1.61 ± 0.54	0.001

Table 2. Mean and Standard Deviation of Echocardiographic Parameters in the Right Heart

Parameter	Case Mean ± SD	Control Mean ± SD	P value
Myocardial performance index	0.58 ± 0.14	0.31 ± 0.04	0.000
Isovolumic relaxation time	0.11 ± 0.02	0.09 ± 0.01	0.001
Acceleration time	0.13 ± 0.19	0.07 ± 0.01	0.060
Deceleration time	0.12 ± 0.01	0.11 ± 0.01	0.041
Peak E Velocity	68.41 ± 17.15	57.70 ± 14.13	0.008
Peak A velocity	50.36±13.85	42.11±9.91	0.006
E/A velocity ratio	1.40 ± 0.47	1.39 ± 0.30	0.908
Pre-ejection period	0.08 ± 0.01	0.07 ± 0.007	0.007
Pre-ejection period/ejection time	0.33 ± 0.05	0.28 ± 0.03	0.000
Ejection time	0.24 ± 0.02	0.26 ± 0.02	0.002
Isovolumic contraction time	0.03 ± 0.01	0.02 ± 0.01	0.095

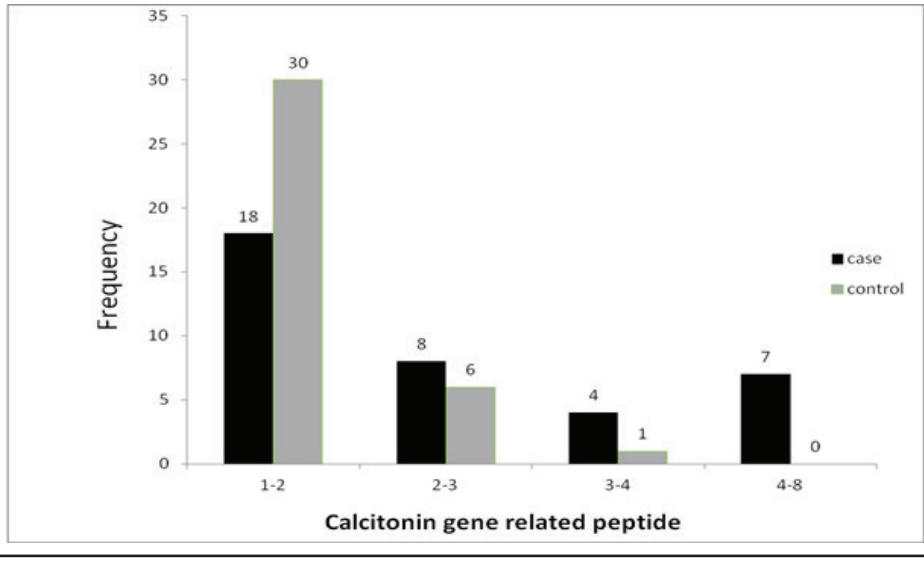

Figure 1. The Frequency of Calcitonin Gene Related Peptide in the Case and Control Groups

Table 3. Accordingly, the majority of echocardiographic parameters were significantly correlated to CGRP in the left heart. In the right heart, on the other hand, CGRP was only associated with one parameter; i.e., MPI.

Table 3. The Correlation between Calcitonin Gene Related Peptide and Echocardiographic Parameters in Left and Right Hearts

Left Heart Parameters	Pearson's Correlation	Sig.
Ventricular end-diastolic dimension	0.211	0.086
Ventricular end-systolic dimension	0.137	0.268
Ejection fraction	0.020	0.871
Fractional shortening	0.111	0.373
Myocardial performance index	0.272	0.026
Isovolumic contraction time	0.115	0.356
Isovolumic relaxation time	-0.092	0.457
Acceleration time	0.099	0.424
Deceleration time	0.033	0.791
Peak E velocity	-0.271	0.026
E/A velocity ratio	-0.018	0.886
Peak A velocity	-0.174	0.159
Atrium aortic ratio	0.001	0.996
Pre-ejection period	0.317	0.009
Pre-ejection period/ejection time	0.245	0.046
Ejection time	-0.083	0.507
Interventricular septal dimension in diastole	0.275	0.024
Ventricular posterior wall dimension in diastole	0.265	0.030
Interventricular septal dimension in systole	0.393	0.001
Ventricular posterior wall dimension in systole	0.287	0.019
Right heart parameters	Pearson's correlation	Sig.
Myocardial performance index	0.311	0.010
Isovolumic relaxation time	0.205	0.097
Acceleration time	-0.068	0.583
Deceleration time	-0.017	0.889
Pre-ejection period	0.156	0.209
Pre-ejection period/ejection time	0.218	0.076
Ejection time	-0.170	0.168
Isovolumic contraction time	0.025	0.843
Peak E Velocity	0.212	0.085
E/A velocity ratio	0.214	0.083
Peak A velocity	0.025	0.843

5. Discussion

This study was conducted on 74 children, 37 healthy children and 37 patients with dilated cardiomyopathy. The children in the two groups were compared with respect to echocardiographic parameters and the echocardiographic findings were compared to CGRP levels. The study results showed that CGRP level was higher among the cases, but it was only correlated to IVSS.

Jianping conducted a study on two different kinds of mice and concluded that LV α-CGRP content, survival rate, echocardiographic parameters, LW/BW ratio, absence of α-CGRP, exacerbated pressure overload-induced cardiac hypertrophy, and inflammation increased fibrosis in the TAC α-CGRP mice and absence of α-CGRP decreased angiogenesis and increased cardiomyocyte apoptosis and necrosis in pressure overloaded hearts. The results of several studies have expressed that CGRP infusion had significant positive clinical effects on patients with heart failure through multiple mechanisms. However, it has been reported that cardiac hypertrophy, inflammation, fibrosis, cell death, and mortality in pressure overloaded hearts exacerbated in the absence of α-CGRP. These results propose that CGRP could be a new treatment for heart failure. The findings of the current study indicated a significant positive correlation between CGRP and LVDD, LVESD, LVPWDD, and LVPWDS, which is comparable with the results of the study conducted by

Jianping in an animal model (rat); however, our study was conducted on humans (11) Marangoni performed an animal study on rats to examine whether development of distal symmetrical polyneuropathy and diabetes was associated with changes in cardiac structure, function, and mechanisms in rats receiving streptozotocin (STZ). They came to the conclusion that LVDD, LVSD, IVSD, IVSS, FS, E/A, IVRT, and DT were higher in the case group compared to the controls at all-time points. In the present study, similar results were obtained regarding IVDD, IVSD, LVSD, IVSS, and IVRT, while the two groups were not significantly different regarding FS, E/A, IVRT, and DT (12). The difference between Marangoni's study and the present one can be due to the subjects under investigation.

By using STZ in mice, a decrease was found in CGRP immunoreactivity and CGRP-specific denervation in Diabetes Mellitus (DM) hearts. Deficits in cardiac sensory afferents, another aspect of DM cardiac pathology that may reflect neuropathy, have been linked to changes in sensory neuronal markers, such as CGRP (13). Another study on female rats demonstrated an increase in CGRP content in DM hearts after using STZ due to accumulation at the dystrophic terminals (14).

The results of the study by Marangoni also showed elevation of myocardial CGRP protein levels and concluded

that cardiovascular structural and functional changes developed early in the course of DM. These results may indicate the need for early cardiovascular evaluation in DM patients with insensate neuropathy (12).

In our study, it seems that increase of serum CGRP levels was due to dysfunction at the nerve terminal with impaired release of neurotransmitters. Hsu JH reported that CGRP level was related to pulmonary artery systolic pressure. They emphasized that heart failure due to CHD and concomitant with elevated pulmonary artery pressure was associated with CGRP. Also, CGRP was related to severity of the disease (5). That study was performed on patients with CHD who had attended for left to right shunt, which can be a cause of pulmonary artery hypertension. Thus, that research is different from ours in terms of cardiomyopathy improvement.

Zhang and colleague showed that CGRP and endothelin plasma levels in patients with CHD were related to the pathophysiological process of pulmonary hypertension and CGRP rose in pulmonary hypertension. These results were comparable with the findings of the present study, indicating higher CGRP levels in the patients with dilated cardiomyopathy (15).

Xin et al. studied the relationship between right heart echocardiographic findings and CGRP in major thalasemia patients and reported that CGRP was inversely correlated to right ventricle MPI and pulmonary hypertension (16). Nonetheless, the current study results showed significant positive correlations in both left and right hearts. Besides, no significant relationship was found between CGRP and MPI in the case group, and there was a direct correlation between IVSS and CGRP level in the left heart.

Another study revealed no correlations between CGRP and endothelin and urotensin II in the patients with CHD and pulmonary hypertension (17). The patients under that study had shunts; therefore, the results were not consistent with ours despite the increase of CGRP in both studies.

Anand evaluated the impact of CGRP on cardiovascular system in animals with heart failure. After CGRP injection, cardiac output increased and vascular resistance decreased. Overall, that study showed that CGRP was a potent vasodilator and could have effects on ventricular myocardium. Thus, it was suggested that CGRP could be used for treatment of heart failure (18). The difference between Anand's study and the present one is in the samples under investigation (animal models instead of humans).

Strecker indicated the beneficial effects of RPV1 on ischemic chest pain in animals. CGRP dysfunction also, through the same mechanism as RPV1 receptors, can be harmful in humans (19). Wang found that the prevalence of coronary vascular events was high in diabetic patients, which might be due to decrease of CGRP and substance P levels. These factors may also have a role in pathogenesis of coronary artery disease (20). The two aforementioned studies were not consistent with our research, because both of them evaluated CGRP dysfunction in coronary artery disease but did not specify its diagnostic value.

Endogenous CGRP has no effects on the cardiovascular dynamic system on the end stage of heart failure. In a study performed on animals in libratory models, heart rate, left atrium, and ventricular pressures was measured. The results of that study demonstrated that CGRP had no effects on the end stage of heart failure (21). That study was conducted on animals and its objective was treatment. Thus, it is different from the current study which involved a diagnostic process among the patients with dilated cardiomypathy.

One other research assessed the impact of CGRP on heart contractility and hemodynamic in idiopathic dilated cardiomyopathy patients. After CGRP injection, norepinephrine increased but blood pressure decreased. Hence, they came to the conclusion that CGRP had no direct effects on heart contractility and its delayed effect was due to vasodilation. Therefore, CGRP was recommended for treatment of heart failure (22). That study was not in line with ours since it dealt with idiopathic dilated cardiomyopathy.

The present research was performed on dilated cardiomyopathy patients and showed that most echocardiographic parameters and the mean of CGRP increased in the case group compared to the control group. The results also indicated a direct relationship between IVSS and CGRP level.

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Authors' Contribution

All the authors contributed to conception and design, analysis and interpretation of the data, and writing, critical revision, and final approval of the manuscript.

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