http:// ijp.mums.ac.ir
Systematic Review (Pages: 5693-5707)

Comparison between Brain Natriuretic Peptide and Calcitonin Gene Related Peptide in Children with Dilated Cardiomyopathy

Noor Mohammad Noori [1], *Alireza Teimouri [1], Iraj Shahramian [2]

[1]Children and Adolescent Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

[2]Department of Pediatric, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.

Abstract

***Background*:** Dilated cardiomyopathy (DCM) is revealed with the left ventricular dilatation and systolic dysfunction. This study was performed to determine the level of Calcitonin Gene Related Peptide (CGRP) and Brain Natriuretic Peptide (BNP) in children with dilated cardiomyopathy and controls and comparison of these two biomarkers in patients.

***Materials and Methods*:** This case-control study was performed from April 2014 to March 2015 on patients with DCM. The levels of BNP and CGRP were measured by ELISA and final amounts of biomarkers were compared with echocardiographic finding. 37 DCM patients compared with 30 healthy children selected randomly from those who referred to the hospital for routine checkup.

***Results*:** In this study the mean age was 10.567± 5.50 and 12.135 ± 4.626 years for controls and cases, respectively (P=0.321). The majority of echocardiography indices in the left and right heart had different means in case and controls (P<0.05). Means of BNP were 213.814 ±309.601 and 2.76 ± 1.013 for case and control groups, respectively (P< 0.001). Means of CGRP were 2.278±1.586 and 1.488±0.501 for case and control groups, respectively (P = 0.001). In the dilated cardiomyopathy children however, no significant relationship was observed between CGRP level and Ross classification, but observed a significant association between Ross classification and BNP (Chi square = 15.85 and P=0.001).

Conclusion: The present research was performed on DCM patients and showed that most echocardiographic parameters, mean of CGRP and mean of BNP increased in patients compared to healthy children. The severity of illness based on the Ross classification showed significant and positive correlation with BNP level but not with CGRP. Probably could be concluded that, BNP would be a better biomarker in DCM patients.

Key Words: Brain Natriuretic Peptide, Calcitonin Gene Related Peptide, Children, DCM.

*Please cite this article as: Noori NM, Teimouri A, Shahramian I. Comparison between Brain Natriuretic Peptide and Calcitonin Gene Related Peptide in Children with Dilated Cardiomyopathy. Int J Pediatr 2017; 5(9): 5693-5707. DOI: **10.22038/ijp.2017.24649.2079**

Corresponding Author:

Alireza Teimouri, M.Phil, Ph.D in Demography, Children and Adolescent Health Research Center, Zahedan University of Medical Sciences, Zahedan- Iran.

Email: Alirezateimouri260@gmail.com

Received date: Jun.13, 2017; Accepted date: Jul. 12, 2017

1- INTRODUCTION

Dilated cardiomyopathy (DCM) is identified with systolic dysfunction and dilatation of left ventricle that may cause ventricular arrhythmia, atrioventricular block, syncope, and finally sudden death. Yearly incidence is 0.57 per 100 000 with the distribution of 0.66 and 0.47 for males and females in children. This term is ranged from 4 to 40 in infants that are much higher than in children (1). Calcitonin Gene Related Peptide (CGRP) neurotransmitter peptide 37 is an amino acid that extracted from calcitonin gene and commonly increases in nervous and cardiovascular systems. A positive inotropic and chronotropic effects of calcitonin has been observed on the heart and it is the strongest endogen vasodilator peptide that has been known so far (2). Inconsistent results reported about CGRP levels in Congestive Heart Failure (CHF) with low information regarding regulation of CGRP function (3-5).

Cardiac sensory nerves play a defensive role in the heart through the expulsion of CGRP and nitric oxide. CGRP plays a protective role in myocardial injuries, including ischemia recirculation and cardio-toxic agent doxorubicin. CGRP in addition of having great power in vasodilator, 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 (6). Brain-type natriuretic peptide (BNP) is a natriuretic hormone secreted from the cardiac ventricular myocytes in response to an increase in ventricular wall stretch and filling pressures. Natriuretic peptides are useful markers in a variety of cardiac diseases in children and adolescents (7). These peptides may be useful for screening of asymptomatic high risk patients such as aged persons, hypertensive, diabetics and coronary artery disease (8). The cut-off point of BNP in children is higher than adults and is a reliable test for diagnosis of functional and structural disorders of the cardiovascular system (9). These peptides are used to diagnose the CHF and may have advantages in screening of asymptomatic high risk patients. BNP is a reliable test for diagnosis of functional and structural disorders of CHD (10). Resulted that CGRP constituted potential therapeutic for the treatment of cardiomyopathy on diabetic rats in long term (11). Moreover, it pointed that CGRP measures are related to the overload pulmonary volume in children with CHD and it can be a cause of CHF and increases with severity (3). The most common cause of CHF is CHD with pulmonary volume overload in infants and children. It also plays an important role in CHF pathogenesis secondary to CHD. Pulmonary volume overload may have a strong role in the regulation of CGRP in children with CHF (3, 5).

In CHF patients, it has been concluded that an increase in BNP level is associated with blood volume increase. Therefore high secretion of BNP is due to pulmonary hypertension and cyanosis. BNP levels are increased in most types of CHD with both volume and pressure overload of left or right ventricles (12). Accordance with a variety in DCM studies and this fact that most of them are about the role of BNP and CGRP in the diagnosis, we decided to perform a study with the aim of using BNP and CGRP to diagnose DCM in children to specify the diagnostic value of these two biomarkers with comparison of control group.

2- MATERIALS AND METHODS

2-1. Study Design and Population

This study was performed on 37 dilated cardiomyopathy patients in one-year duration from April 2014 to March 2015. Thirty healthy children selected randomly from those who referred to the hospital for routine checkup and matched in age and

weight with patients. Participants aged from 1 to 18 years. Accordance to Ross classification, the patients were grouped in 4 categories as: group I that deals to patients with no limitations or symptoms, group II that deals to patients with mild tachypnea or diaphoresis with feedings in infants, dyspnea at exertion in older children; no growth failure, group III that deals to patients who marked tachypnea or diaphoresis with feedings or exertion and prolonged feeding times with growth failure from CHF and group IV that deals to patients with symptomatic at rest with tachypnea, retractions, grunting, or diaphoresis

2-2. Inclusion and exclusion criteria

Hemoglobin less than 10 g/dl, endocrine, metabolic, valvular disorders, dysrhythmia and heart block were exclusion criteria. For the control participants if echocardiography diagnosed any types of heart diseases, they were excluded from the study and replaced with new one based on conditions.

2-3. Measurements

Children over 2 years measured weight using RASA Mark made in Iran by an error of 100g and for children under 2 years, MIKA Mark recumbent weighting scale made in Japan with error of 10g was applied. Height of patients was measured in the recumbent position by using a calibrated and flat wooden table, while that of the children above 2 years old was measured in the standing position with a scale ruler. Heart failure stratification was performed by modified Ross classification. Patients were normal in respect to Na, K, Ca, blood nitrogen urea and creatinine. Three milliliters blood was drawn from the patients in fasting at 8 AM. Samples were centrifuged at 5 °C with a round of 3000g for 10 minutes. The separated serum was kept in -80°C refrigerator. The levels of BNP and CGRP were measured by ELISA kits. Echocardiography findings were measured by My Lab 60 made in Italy.

2-4. Ethical considerations

All the participants or their parents received written information about the aims of the study. Consent letter gave them for taking agreement. The study was in accordance with the ethical standards for human experimentation and approved by the Research Deputy (RD) ethics committee of the ZaUMS, Iran.

2-5. Data analysis

Data analysis was performed by SPSS version 20.0 software using test of normality at first with Kolmogorov-Smirnov. In the case of normality, independent t-test and one way ANOVA were used otherwise same non-parametric tests such as Mann-Whitney U and Kruskal-Wallis H. For the relationship between variables, Pearson correlation was used. In all test the significant level considered less than 0.05.

3- RESULTS

In this study 67 children were recruited in case (37) and control (30). The age range was from 1 to 18 years. The mean age was 11.933± 7.625 and 13.568± 6.517 years for control and case groups, respectively (t=0.946, P=0.348). The mean weight was 35.133 ± 18.471 and 28.7 02± 10.553 kg in control and case groups, respectively (t= -1.79, P=0.078). The mean height was 128.700 ± 29.200 and 127.405 ± 18.712 cm in control and case groups, respectively (t= -0.22, P=0.827).

Since the sample size is larger than 50 we use the Kolmogorov-Smirnov normality test for variables in the study. The null hypothesis for the test of normality stated that the actual distribution of the variable is equal to the expected distribution, i.e., the variable is normally distributed. Since the probability associated with the test of

normality for all variables except weight, left ventricular end-diastolic volume (LVEDV), fractional shortening (FS), left peak E velocity, body mass index (BMI) and right isovolumic contraction time (ICT), is less than or equal to the level of significance (0.05), we reject the null hypothesis and conclude that these variables are normally distributed. Therefore for these variables all applied tests would be parametric.

Table.1 shows the results of independent t- test to compare the means of normal distributed echocardiographic parameters of left and right heart in case and control groups. The results showed that mean LVEDV for case and control groups was 66.108±22.129 and 39.279±14.782 with significant level in different (t=-5.687, P<0.001), and same trends for means of case and control for left peak E velocity with 85.974±17.759 and 101.558±19.648, respectively (t=-3.406, P=0.001).

An examination of the findings in **Tables 2 and 3** shows that the results of the non-parametric Mann Whitney U- test applied to the variables in left and right in the case and control groups revealed a statistically significant difference at the level in some of them. The rank average of the left ventricular end-diastolic dimension (LVEDD) of the case group was 42.568, while the participants in the control group had an LVEDD score rank average of 23.433 with the P ≤0.001 which shows statistically significant different (**Table.4**).

The mean ranks of the right E/A velocity ratio were 33.216 and 34.967 in the case and control groups, respectively with the P = 0.714 in which not enough power to reject equality of mean ranks (**Table.5**). Note that the test statistic was corrected for the existence of ties in the ranks of the BNP (Chi-square = 15.845, P ≤0.05) and CGRP (Chi-square = 297, P ≤0.05) data there exists enough evidence to result that there is a difference in the Mann-Whitney test scores. **Table.4** shows Ross classification impact on BNP and CGRP in patients. CGRP with mean ranks of 20.95, 20.56, 18.25 and 15.75 for severity of classes 1, 2, 3 and 4 in the order given shows not significant relationship with Ross classification. However according to the **Table.4**, BNP shows that influenced by the level of disease's severity significantly (Chi-square =15.8453 and P= 0.001).

Table.5 shows that with BNP increasing CGRP decreases but not significantly in bout patients and all population. LVEDV had appositive and significant correlation (r=0.447, P= 0.000) with BNP in the case of considering all population but not with CGRP. It is observed that there was no significant correlation in the case of patients except a few parameters. In patients observed that just interventricular septal dimension in systole (IVSDs) had a correlation with CGRP.

At the end of the analysis, from 10 patients in group I, two patients died due to disease progression. All nine participants in the second groups survived until the end of follow up. In group III, one patient died and one had heart transplantation (from 10 patients). And finally, for the fourth group, from eight patients, two received heart transplantation, one with three chambers peacemaker (died), and two individuals withdraw the study (*Please see the all tables at the end of paper*).

4- DISCUSSION

The Current study provided that LVEDD, left ventricular end-systolic dimension(LVESD), ICT, LVEDV, myocardial performance index (MPI), ICT, IRT,IVSDS, peak E velocity, pre ejection period/ejection time (PEP/ET), ET, interventricular septal dimension in diastole (IVSDD) , left ventricular posterior wall dimension in diastole (LVPWDD), and left ventricular posterior wall dimension in systole (LVPWDS) in the left heart and MPI, IRT, deceleration

time (DT), E, A (peak A velocity), PEP/ET, PEP, and ET in the right heart were significantly different between children with dilated cardiomyopathy and controls. The results showed that CGRP and BNP levels were higher in patients, but it was very stronger significant in BNP. Increased BNP may approve the presence of DCM due to diastolic dysfunction and probably systolic dysfunction as well but without differentiation power between systolic and diastolic types. Noori et al. in a study concluded a significant difference in the levels of IRT, DT, PEP, ET, PEP/ET and MPI of the right heart between children with dilated cardiomyopathy and controls. For the other echocardiography parameters such as ICT, E, A and E/A non-significant difference was observed which is similar with the present findings in all echocardiography parameters except peak E velocity and peak A velocity in the left heart. In our study we resulted that the findings comparatively is similar with the Noori's outcomes (13).

Noori carried out studies on the cardiac and pulmonary dysfunction in asymptomatic beta thalassemia major patients. In the studies the right and left side heart functions compared in patients with thalassemia major, patients with thalassemia intermedia and control groups, resulted that MPI in both right and left heart was significantly higher in patients than controls which was similar with our finding in this specific situation (14 , 15). PEP/ET ratio was different in case and controls significantly with similar outcome of the present study. Ejection fraction, in both studies showed a significant difference that was similar with the present study. For acceleration time in left and right heart was not reported a significant difference. Mean of ICT as a parameter of left heart was different in case and control comparison significantly which was similar with our findings. Isovolumic relaxation time (IRT) was significantly different in both studies. In the present study we received to the conclusion of a significant difference in both left and right heart. DT had significant difference in two case and control groups in Noori et al.'s studies that agree with right heart. In brief, these two studies by Noori et al. show similar patterns with the results of the present study (14, 15). Left ventricular mass index (LVMI) as a left heart parameter had significant different values in case and control groups (16, 15). The results of the late studies were similar with the present study findings. Noori et al. reported that echocardiographic parameters of left ventricle such as ICT, MPI, ET, PEP/ET, DT, AT, IRT, LVEDD, LVESD and EF in patient's and control groups were significantly different. However in the present study the results were a little dissimilar. It means that, for all the mentioned parameters we found same results except DT, AT and EF. Echocardiographic parameters of right ventricle such as MPI (higher in patient) peak A velocity (higher in patient), PEP (higher in patient), and ET (higher in case) in patients were statistically different compared with controls which was similar with our findings. However for the E/A (higher in case) parameter we found disagreement with the Noori et al. study which was not significantly different in case and controls (17).

Jefferies and Towbin reported that DCM is characterized with left ventricular dilatation and is accompanied with systolic dysfunction. However diastolic dysfunction may also be seen and both ventricles may develop insufficiency and also there is mortality risk due to ventricular arrhythmia, atrioventricular block, syncope, and sudden death (1). In our patients, we received to the conclusion that BNP had strong relationship with three of echocardiographic parameters and severity of illness which was consisted

with Jefferies' results. NT-pro-BNP could be introduced for continuous left ventricle dysfunction in children with cardiomyopathy or myocarditis. However the normal level in improved children may not demonstrate any residual heart disease (9). This result was similar to our findings comparatively when we used BNP instead of NT-pro-BNP. Consequently, to diagnose the heart failure BNP has been approved by FDA. Similarly we found an association between BNP and MPI that showed systolic and diastolic dysfunction (18). Koch et al. demonstrated that in children with congenital heart disease, the plasma level of BNP was associated with ventricular function and reflects the ventricular overload injury. Hence the normal BNP may show compensatory phase (19).

Our results were similar with Koch when we assessed children with DCM. In both studies the major problem was due to volume overload. Mariano-Goulart et al. reported that an increase in BNP level in patients with systolic ventricular dysfunction would be considered as a risk factor for right ventricular dysfunction (20). According to our finding, the BNP level was higher in high severity level of disease based on Ross classification. Kremastinos et al. said in their report "plasma level of BNP and NT-Pro-BNP is increased in major thalassemia patients and when left ventricular dysfunction is developing the predictive value of NT-pro-BNP would be better than BNP for diagnosis of latent left ventricular diastolic dysfunction". Moreover in the related study by Kremastinos et al. they were also proposed that NT-pro-BNP was increasing by age (21, 22). In our study a significant difference in mean age and BNP level was observed in the various severity of disease such that, in higher severity the mean age and the mean BNP were higher. Niedner et al. found a relationship between BNP and severity of congenital heart disease (23) however in our study we resulted that increasing of BNP was correlated with severity of DCM. The results of various studies on CGRP have reported that CGRP infusion had meaningful positive clinical impacts on patients with heart failure during multiple mechanisms. These results proposed that heart failure would be treated by CGRP. The findings of the present study showed a significant positive correlation between CGRP and LVEDD, LVESD, LVPWDD, and LVPWDS, which was comparable with the results of the study conducted by Jianping in animal model (24). Marangoni et al. conducted an animal model study on rats and resulted that FS, E/A, IVRT, LVDD, LVSD, IVSD, IVSS, and DT were higher in the cases compared with controls. In the current study, similar results were obtained regarding IVDD, IVSD, LVSD, IVSS, and IVRT, while the two groups were not significantly different in respect to FS, E/A, IVRT and DT (25).

Hsu et al. expressed that CGRP level was related to pulmonary artery systolic pressure's reported an association between CGRP and heart failure due to CHD and pulmonary artery pressure. Hsu et al. also pointed CGRP was related to the severity of the disease (3). A study was carried out on CHD patients who had left to right shunt by Zhang et al. The research was different with ours in terms of cardiomyopathy improvement (26).

They measured CGRP and endothelin plasma levels in CHD patients with pulmonary hypertension. They resulted that the level of CGRP increased in patients with pulmonary hypertension. These results were comparable with our findings for showing higher CGRP levels in the patients with DCM (26). Xin et al. studied the relationship between right heart echocardiographic findings and CGRP in major thalassemia patients and reported that CGRP was inversely correlated with right ventricle MPI and pulmonary

hypertension however in another study resulted that, there were no correlations between CGRP and pulmonary hypertension in CHD patients (27, 28). 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.

4-1. Limitation of study

The study limitation was lack of properly corporation by participants, especially in control group.

5- CONCLUSIONS

The present research was performed on DCM patients and showed that the majority echocardiographic parameters, mean of CGRP and mean of BNP increased in patients compared to healthy children. The severity of illness based on the Ross classification showed significant and positive correlation with BNP level but not with CGRP. Probably could be concluded that BNP would be a better biomarker in DCM patients.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENTS

The authors would like to show their warm gratitude to all parents for the participation especially control groups. The authors also thank to nursing staff from hospitals for data gathering.

8- REFERENCES

1. Jefferies JL, Towbin JA. Dilated cardiomyopathy. Lancet 2010; 375: 752–62.

2. Noori N, Shahramian I, Mahjoobifar M, Teymoori A, Shahraki Z. Comparison of calcitonin gene related peptide level between children with dilated cardiomyopathy and control group. International Cardivascular Research Journal 2015; 9(2):100-5.

3. Hsu JH, Yeh JL, Dai ZK, Chen IJ, Wu JR. Increased circulating calcitonin gene-related peptide in congestive heart failure caused by congenital heart disease. Int Heart J. 2005; 46(5):867-75.

4. Spinarova L, Vitovec J. Neurohumoral changes in chronic heart failure. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2007; 151(2):201-7.

5. Hasbak P, Saetrum Opgaard O, Eskesen K, Schifter S, Arendrup H, Longmore J, et al. Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with a nonpeptide antagonist. J Pharmacol Exp Ther. 2003; 304(1):326-33.

6. Boerma M, Hauer-Jensen M. Preclinical research into basic mechanisms of radiation-induced heart disease. Cardiology Research and Practice Volume 2011, Article ID 858262, 8 pages doi:10.4061/2011/858262.

7. Noori N, Teimouri A, Miri- Aliabad G. Brain Natriuretic Peptides and Calcitonin Gene-Related Peptide in Diagnosis of Cardiac Involvement in Major Thalassemia Patients. Iranian Journal of Pediatric Hematology and Oncology 2017; 7 (1): 25-36.

8. Noori NM, Teimouri A, Anvari N. Diagnostic Value of N Terminal Pro B Type Natriuretic Peptide (NT-pro BNP) in Cardiac Involvement in Patients with Beta-Thalassemia. International Journal of Pediatrics. 2017;5(4):4641-62.

9. Noori NM, Teimouri A, Shahramian I, Akhavan Sales S. Evaluation of brain natriuretic peptide plasma levels in children with congenital heart diseases. International Journal of Pediatrics 2016; 4(10):3615-26.

10. Noori NM, Maziar Mahjoubifard, Iraj Shahramian, Alireza Teimouri, and Alireza Jahangirifard. Comparison between Procalcitonin, Brain Natriuretic Peptide, and Uric Acid in Children with Cardiomyopathy and Controls. BioMed Research International Volume 2015, Article ID 510450, 7 pages http://dx.doi.org/10.1155/2015/510450.

11. Dvorakova MC, Kruzliak P, Rabkin SW. Role of neuropeptides in cardiomyopathies. Peptides. 2014; 61:1-6.

12. Trojnarska O, Gwizdała A, Katarzyński S, Katarzyńska A, Szyszka A, Lanocha M, et al.

Evaluation of exercise capacity with cardiopulmonary exercise test and B-type natriuretic peptide in adults with congenital heart disease. Cardiology journal 2009; 16(2):133-41.

13. Noori NM, Rajaei S. Serum uric acid correlation with echocardiographic indices in children with dilated cardiomyopathy. The Journal of Tehran University Heart Center. 2009; 4(4):230-3.

14. Noori NM, Kambiz Keshavarz. Mosaeib Shahriar. Cardiac and pulmonary dysfunction in asymptomatic beta-thalassanemia major. Asian Cardiovasc Thorac Ann. 2012; 20(5):555-9. doi: 10.1177/0218492312439706.

15. Noori NM, Mohamadi M, Keshavarz K, Alavi SM, Mahjoubifard M, Mirmesdagh Y. Comparison of Right and Left Side Heart Functions in Patients with Thalassemia Major, Patients with Thalassemia Intermedia, and Control Group. J Teh Univ Heart Ctr 2013; 8(1):35-41.

16. Noori NM, Mahjoubifard M, Mohammadi M, Jahangiri Fard A, Abassi A, Farzanegan B. Comparison of QT Dispersion with Left Ventricular Mass Index in Early Diagnosis of Cardiac Dysfunction in Patients With β-Thalassemia Major. Iran Red Crescent Med J. 2014; 16(5): e11698. DOI: 10.5812/ircmj.11698.

17. Noori NM, Semira Mehralizadeh. Echocardiographic evaluation of systolic and diastolic heart function in patients suffering from beta-thalassemia major aged 5-10 years at the Zahedan Research Center for Children and Adolescent Health. Anadolu Kardiyol Derg 2010; 10: 150-3.

18. Bhatia V, Nayyar P, Dhindsa S. Brain natriuretic peptide in diagnosis and treatment of heart failure. J Postgrad Med 2003; 49:182-5.

19. Koch A, Zink S, Singer H. B-type natriuretic peptide in pediatric patients with congenital heart disease. Eur Heart J. 2006; 27, 861-6.

20. Mariano-Goulart D, Eberlé MC, Boudousq V, Hejazi-Moughari A, Piot C, Caderas de Kerleau C, Verdier R, et al. Major increase in brain natriuretic peptide indicates right ventricular systolic dysfunction in patients with heart failure, Eur J Heart Fail. 2003; 5: 481-8.

21. Kremastinos DT ,Hamodraka E, Parissis J, Tsiapras D, Dima K. Predictive value of B-type natriuretic peptides in detecting latent left ventricular diastolic dysfunction in β-thalassemia major. Am Heart J. 2010; 159(1): 68-74.

22. Kremastinos DT, Tsiapras DP, Kostopoulou AG, Hamodraka ES, Chaidaroglou AS, Kapsali ED. NT-proBNP levels and diastolic dysfunction in β-Thalassaemia major patients. Eur J Heart Fail. 2007; 9: 531-6.

23. Niedner MF, Foley JL, Riffenburgh RH, Bichell DP, Peterson BM, Rodarte A. B-type natriuretic peptide: perioperative patterns in congenital heart disease. Congenit Heart Dis. 2010; 5(3):243-55.

24. Li J, Levick SP, DiPette DJ, Janicki JS, Supowit SC. Alpha-calcitonin gene-related peptide is protective against pressure overload-induced heart failure. Regul Pept. 2013; 185: 20-8.

25. Marangoni MN, Brady ST, Chowdhury SA, Piano MR. The co-occurrence of myocardial dysfunction and peripheral insensate neuropathy in a streptozotocin-induced rat model of diabetes. Cardiovasc Diabetol 2014; 13:11.

26. Zhang ZW, Lin R, Li JH, Hu J, Wang X. Perioperative changes in plasma endothelin and calcitonin gene-related peptide in congenital heart disease with pulmonary hypertension. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2003; 32(3):212-4.

27. XIN P, SUN M, SUN G, DING H, ZHU W, WU Z, et al. The relationship of the calcitonin gene-related peptide and the right ventricular function in patients with pulmonary hypertension. Journal of Youjiang Medical College for Nationalities 2005; 1: 001.

28. Fang W, Chen H, Huang J, Luo Z, Huang J. Clinical Observation of Significance of Urotensin Ⅱ, Calcitonin Gene Related Peptide and Endothelin in Patients with Congenital Heart Disease Complicated with Secondary Pulmonary Hypertension [J]. Chinese Journal of Extracorporeal Circulation 2008; 3: 011.

Table-1: Echocardiographic parameters of left and right heart

Parameters	Group	Mean	SD	t-test	P-value
Left Ventricular end-diastolic volume (ml)	Case	66.10811	22.12908	5.687	0.000
	Control	39.27867	14.78177		
Fractional shortening (%)	Case	35.40541	8.067285	1.2972	0.199
	Control	33.33333	3.735508		
Left Peak E Velocity (cm/s)	Case	85.97405	17.75868	-3.4057	0.001
	Control	101.5586	19.64822		
Left Peak A Velocity (cm/s)	Case	55.37649	15.50389	-0.5643	0.574
	Control	57.54533	15.81358		
Right Isovolumic contraction time (ms)	case	0.034	0.016969	1.6933	0.095
	control	0.0272	0.015535		

SD: Standard deviation.

Table-2: Results of the Mann Whitney U Test to Compare the Groups' values of left heart parameters

Parameters	Group	Mean	SD	Mean Rank	Sum of Ranks	Mann-Whitney U test value	P-value
Height (cm)	Case	127.405	18.712	32.797	1213.5	510.5	0.574
	Control	128.7	29.2	35.483	1064.5		
Left Ventricular end-diastolic dimension (mm)	Case	46.584	7.713	42.568	1575	238	0.000
	Control	39.993	4.098	23.433	703		
Left ventricular end-systolic dimension (mm)	Case	30.181	7.451	38.716	1432.5	380.5	0.028
	Control	26.517	3.23	28.183	845.5		
Ejection Fraction (%)	Case	62.514	13.715	35.986	1331.5	481.5	0.353
	Control	63.533	5.507	31.55	946.5		
Left myocardial performance index (-)	Case	0.549	0.181	45.892	1698	115	0.000
	Control	0.325	0.051	19.333	580		
Left Isovolumic contraction time (ms)	Case	0.03	0.01	43.865	1623	190	0.000
	Control	0.017	0.007	21.833	655		
Left Isovolumic relaxation time (ms)	Case	0.107	0.026	43.23	1599.5	213.5	0.000
	Control	0.094	0.012	22.617	678.5		
Left acceleration time (ms)	Case	0.054	0.011	33.703	1247	544	0.888
	Control	0.054	0.008	34.367	1031		
Left deceleration time (ms)	Case	0.123	0.014	35.581	1316.5	496.5	0.456
	Control	0.121	0.015	32.05	961.5		
Left E/A velocity ratio (-)	Case	1.657	0.757	28.757	1064	361	0.014
	Control	1.842	0.461	40.467	1214		
Left atrium/ aorta ratio (-)	Control	1.252	0.294	33.662	1245.5	542.5	0.875
	Case	1.203	0.15	34.417	1032.5		

Left Pre-ejection period (ms)	Control	0.128	0.156	44.703	1654	159	0
	Case	0.094	0.101	20.8	624		
Left Pre-ejection period/ejection time (-)	Control	0.357	0.058	44.743	1655.5	157.5	0
	Case	0.294	0.039	20.75	622.5		
Left Ejection time (ms)	Control	0.25	0.026	27.865	1031	328	0.004
	Control	0.263	0.02	41.567	1247		
Left Interventricular septal dimension in diastole (mm)	Case	6.319	1.294	40.243	1489	324	0.003
	Control	5.43	0.887	26.3	789		
Left Ventricular posterior wall dimension in diastole (mm)	Case	4.211	1.222	41.149	1522.5	290.5	0.001
	Control	3.343	0.507	25.183	755.5		
Left Interventricular septal dimension in systole (mm)	Case	9.276	1.761	39.365	1456.5	356.5	0.012
	Control	8.283	1.08	27.383	821.5		
Left Interventricular septal dimension in systole (mm)	Case	4.251	1.246	41.662	1541.5	271.5	0.000
	Control	3.363	0.524	24.55	736.5		
Left Ventricular Mass Index (g/m^2)	Case	27.0069	10.64840	43.92	1625.00	188.000	0.000
	Control	16.4649	5.47652	21.77	653.00		

SD: Standard deviation.

Table-3: Results of the Mann Whitney U Test to Compare the Groups' values of right heart parameters and biomarkers

Parameters	Group	Mean	SD	Mean Rank	Sum of Ranks	Mann-Whitney U test value	P-value
Height (cm)	Case	127.405	18.712	32.797	1213.5	510.5	0.574
	Control	128.7	29.2	35.483	1064.5		
myocardial performance index (-)	Case	0.582	0.147	47.378	1753	60	0.000
	Control	0.316	0.05	17.5	525		
Right Isovolumic relaxation time (ms)	Case	0.11	0.022	40.176	1486.5	326.5	0.004
	Control	0.096	0.012	26.383	791.5		
Right acceleration time (ms)	Case	0.134	0.197	30.068	1112.5	409.5	0.063
	Control	0.07	0.019	38.85	1165.5		
Right deceleration time (ms)	Case	0.128	0.012	38.689	1431.5	381.5	0.027
	Control	0.12	0.018	28.217	846.5		
Right Peak E velocity (cm/s)	Case	68.413	17.158	40.203	1487.5	325.5	0.004
	Control	57.706	14.139	26.35	790.5		
Right E/A velocity ratio (-)	Case	1.408	0.478	33.216	1229	526	0.714
	Control	1.397	0.307	34.967	1049		
Right Peak A velocity (cm/s)	Case	50.366	13.852	40.203	1487.5	325.5	0.004
	Control	42.119	9.916	26.35	790.5		
Right Pre-ejection period (ms)	Case	0.085	0.015	43.054	1593	220	0
	Control	0.076	0.008	22.833	685		
Right Pre-ejection period/ejection time (-)	Case	0.34	0.06	45.973	1701	112	0
	Control	0.289	0.03	19.233	577		
Right Ejection time (ms)	Case	0.246	0.021	27.365	1012.5	309.5	0.002
	Control	0.264	0.024	42.183	1265.5		

CGRP (pg/ml)	Case	2.2779	1.58587	40.97	1516.00	297	0.001
	Control	1.4478	.50102	25.40	762.00		
Brain Natriuretic Peptide (pg/ml)	Case	213.814	309.601	48.419	1791.5	21.5	0.000
	Control	2.76	1.013	16.217	486.5		
Age (year)	Case	12.135	4.626	36.122	1336.5	476.5	0.321
	Control	10.567	5.5	31.383	941.5		

SD: Standard deviation.

Table-4: Ross classification impact on biomarkers in patients

Parameters	DCM	Mean	SD	Number	Mean Rank	Chi-Square	P-value
Calcitonin gene-related peptide	1	3.2500	2.34438	10	20.95	1.29	0.733
	2	2.9556	1.94429	9	20.56		
	3	2.5100	1.50366	10	18.25		
	4	2.4875	2.06566	8	15.75		
Brain Natriuretic Peptide	1	97.130	155.2863	10	12.7500	15.85	0.001
	2	76.656	76.4345	9	14.2778		
	3	133.050	102.7770	10	19.4500		
	4	614.925	452.6220	8	31.5625		

DCM: Dilated Cardiomyopathy; SD: Standard deviation.

Table-5: Correlation of Echocardiographic parameters and biomarkers in patients

Echocardiographic Parameters	Patients population			
	Calcitonin gene-related peptide		Brain Natriuretic Peptide	
	Pearson Correlation	P-value	Pearson Correlation	P-value
Left Ventricular end-diastolic volume (mm)	-0.180	0.287	0.322	0.052
Left Ventricular end-diastolic dimension (mm)	0.079	0.642	-0.057	0.737
Left ventricular end-systolic dimension (mm)	0.057	0.738	-0.136	0.422
Ejection Fraction (%)	0.035	0.836	0.118	0.487
Fractional shortening (%)	0.052	0.762	0.101	0.551
Left myocardial performance index (ms)	0.041	0.809	-0.268	0.108
Left Isovolumic contraction time (ms)	-0.170	0.315	0.305	0.066
Left Isovolumic relaxation time (ms)	-0.251	0.134	-0.408	0.012
Left acceleration time (ms)	0.096	0.572	-0.035	0.839
Left deceleration time (ms)	-0.043	0.803	0.049	0.774
Left Peak E Velocity (cm/s)	-0.172	0.308	0.360	0.028
Left E/A velocity ratio (cm/s)	0.049	0.774	0.277	0.097
Left Peak A Velocity (cm/s)	-0.214	0.203	0.012	0.942
Right myocardial performance index (-)	0.034	0.841	0.188	0.264
Right Isovolumic relaxation time (ms)	0.076	0.656	0.008	0.962
R acceleration time (ms)	-0.168	0.319	-0.021	0.904
R deceleration time (ms)	-0.174	0.304	-0.106	0.531
Right Peak E Velocity (cm/s)	0.145	0.393	-0.154	0.362
Right E/A velocity ratio (-)	0.284	0.089	-0.068	0.689
Right Peak A Velocity (cm/s)	-0.130	0.442	-0.123	0.469

Left atrium/ aorta ratio (-)	-0.062	0.716	0.059	0.727
Left Pre-ejection period (ms)	0.300	0.072	-0.150	0.374
Left Pre-ejection period/ejection time (-)	0.003	0.987	-0.069	0.684
Left ejection time (ms)	0.071	0.675	0.119	0.483
Right Pre-ejection period (ms)	0.061	0.721	0.035	0.839
Right Pre-ejection period/ejection time (-)	0.058	0.735	0.020	0.905
Right ejection time (ms)	-0.002	0.992	0.017	0.923
Left Interventricular septal dimension in diastole (mm)	0.210	0.212	-0.112	0.508
Left Ventricular posterior wall dimension in diastole (mm)	0.141	0.405	-0.161	0.342
Left Interventricular septal dimension in systole (mm)	0.375	0.022	0.008	0.962
Left Ventricular posterior wall dimension in systole (mm)	0.170	0.314	-0.117	0.491
Right Isovolumic contraction time (ms)	-0.059	0.730	-0.271	0.105
Body Mass Index (g)	0.028	0.870	0.120	0.478
Left Ventricular Mass Index (g/m^2)	0.188	0.266	-0.120	0.479