

## Brain Natriuretic Peptides and Calcitonin Gene-Related Peptide in Diagnosis of Cardiac Involvement in Major Thalassemia Patients

Noormohammad Noori MD<sup>1</sup>, Alireza Teimouri PhD<sup>2</sup>, Ghasem Miri- Aliabad MD<sup>3</sup>

1. Professor of Pediatric Cardiology, Children and Adolescents' Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

2. Assistant professor of Demography, Children and Adolescents' Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

3. Assistant professor of Pediatric Hematology and Oncology, Children and Adolescents' Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

\*Corresponding author: Alireza Teimouri, PhD. Assistant professor of Demography Children and Adolescents' Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran. [E-mailAlirezateimouri260@gmail.com](mailto:E-mailAlirezateimouri260@gmail.com).

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### Abstract

**Background:** Cardiac dysfunction is one of the major causes of morbidity and mortality in thalassemia patients. This study aimed to compare the effect of Brain Natriuretic Peptides (BNP) and Calcitonin Gene-Related Peptide (CGRP) with echocardiographic findings in early diagnosis of cardiac disease in major thalassemia patients.

**Materials and Methods:** This study was performed on 80 patients among 500 major thalassemia patients and 80 healthy people. Those with metabolic, endocrine disorder, hypertension, heart failure, and valvular disease excluded from the study. These two groups were matched based on age and sex. Essential heart findings were analyzed using Mylab 60. After blood sampling, levels of CGRP and BNP were measured by use of ELISA kit from extracted plasma. Mann-Whitney test, independent t-test, and Pearson correlation were used to analyze data and  $P < 0.05$  was considered significant.

**Results:** The results showed that mean age of all participants was  $17.581 \pm 5.344$  years when distributed between case and control as  $18.21 \pm 5.14$  and  $16.95 \pm 5.49$  respectively without significant difference. Means of weight, length, hemoglobin, systolic and diastolic pressure were lower in patients group. The majority of echocardiography findings of left and right heart were higher in case group. The average of CGRP and BNP level were more in case group ( $p < 0.05$ ). A positive correlation was observed between BNP ( $R = 0.229$ ,  $P = 0.041$ ) and right MPI. There was also correlation between PEP ( $R = 0.0238$ ,  $P = 0.035$ ), PEP/ET ( $P = 0.005$ ) of right heart and peak A velocity of left heart ( $R = 0.245$ ,  $P = 0.03$ ) with CGRP.

**Conclusion:** Findings of this survey showed that systolic and diastolic function of left heart would be changed in patients with major beta thalassemia. Therefore, monitoring BNP and CGRP in symptom free thalassemia patients as well as serial echocardiography is recommended.

**Keywords:** Brain Natriuretic Peptides, Calcitonin Gene-Related Peptide, Children, Echocardiography, Major thalassemia rt

### Introduction

Thalassemia major is a common genetic disorder that causes severe anemia from early childhood. Annually 60000 to 100000 livebirth are born with thalassemia major worldwide. During the last three decades, the treatment of patients with multiple blood transfusions and systemic iron chelating agents has caused a significant improvement in the quality of life and life expectancy (1-3). Cardiac involvements are important causes of

death in patients with major thalassemia. Iron overload is a reason for permanent and severe cardiac involvement especially for untreated anemia (2). Two broad spectrum of the thalassemia range from asymptomatic carriers to patients with dependent transfusion thalassemia major. Chronic hypoxia and increased pulmonary with systemic vascular resistance are main factors of leading to heart disease in thalassemia. The cause of increased

pulmonary vascular resistance in patients with beta thalassemia is multi-factorial(2). Systolic and diastolic dysfunctions that following hemosiderosis are main causes of early death for these patients(3).

In the other hand, the most common cause of death in major thalassemia patients is dilated cardiomyopathy accompanied by left ventricular dysfunction (4). Neurohormones are hormones which produced and released by neuroendocrine cells into the blood and Brain Natriuretic Peptides (BNP) is one of them that released from ventricular myocytes response to ventricular dysfunction and wall stress. Introduced BNP is broken to NT-Pro BNP in circulation(5). BNP and NT-pro-BNP are extensively used for heart failure diagnosis. These peptides may be useful for screening asymptomatic high risk patients such as patients with hypertension, diabetes mellitus, coronary artery disease, and thalassemia(6). A plasma BNP decrease is occurring in the first week of life (7). The level of NT-Pro BNP is increasing in major beta thalassemia patients compared to healthy. This increase is due to age and left ventricular dysfunction and it is a biomarker to determine the left ventricular dysfunction compared with doppler echocardiography in thalassemia patients (8). CGRP has positive inotropic and chronotropic effects on the heart and is also considered the most potentially endogenous peptide. Previous reports on the changes of CGRP in congestive heart failure (CHF) are varied and poorly spoken about regulation of CGRP production(9). Unfortunately, inconsistent reports are exist about the effects of CGRP on CHF with various conditions that increased the levels of CGRP such as pregnancy, hepatic cirrhosis or hemodialysis (5). However, in infants and children the most common etiology of CHF is CHD and increasing pulmonary blood flow plays an important role in the pathogenesis in CHF to secondary CHD. CGRP is the most important vasodilator with positive

inotropic effects and the most potent vasoconstrictor in vessels (9,10). In hemodialysis patients, the plasma levels of CGRP are significantly higher in patients with fluid excess and are correlated with the degree of fluid overload (9-10). The level of calcitonin of plasma increases in left to right shunts and it would be a reason of moderating the severe of pulmonary hypertension (10). Because the number of investigations in the role of CGRP and BNP in diagnosis cardiac involvements in thalassemia patients is low, the present study aimed to assess the role of CGRP and BNP in early diagnosis of cardiac involvement in asymptomatic major thalassemia patients with respect to control group.

### **Materials and Methods**

This study was performed on 80 patients in age ranged of 10-25 years selected randomly among 500 major thalasemic patients who referred to Aliasghar center during March 2014 to March 2015. Same numbers were selected from healthy people who referred to the center for routine checkup. Sample size was based on the cost, age, and many ethical limitations for controlling participants .These two groups of participants were matched in terms of age and sex to reduce the impact of probably interpositions. Major proceedings were performed on both case and control groups such as medical history, physical examination, chest X-Ray, and Electrocardiogram (ECG) before echocardiography as well Echocardiography was performed by the same cardiologist. Essential parameters which had been analyzed in both groups were as follows: Deceleration time(DT) ,Myocardial Performance Index(MPI) ,peak E velocity/peak A velocity (E/A),Isovolemic contraction time(ICT), Isovolemic relaxation time(IRT), Interventricular septal dimension in systole(IVSS), Interventricular septal dimension in diastole(IVSD),Left ventricular end-diastolic

dimension(LVDD), Fractional Shortening (FS), Ejection Fraction(EF), Left ventricular end-systolic dimension(LVSD), Left ventricular posterior wall dimension in diastole(LVPWD), Left ventricular posterior wall dimension in systole(LVPWS), Acceleration Time(AT), Left ventricular mass index(LVMI). From all participants, those who had metabolic disorders, endocrine disorders, hypertension, heart failure, and valvular disease excluded from the study. Echocardiography was performed on patients 48-72 hours following packed red blood cell transfusion by same pediatric cardiologist using My lab 60 with transducer 3-8 made in Italy. For more precision echocardiography was repeated in 3 different cycles of 2D, M-Mode, and Doppler methods and considered the average. Echocardiogram applied in supine position and without breath holding and view was taken in the mitral valve surface in parasternal position. Systolic and diastolic interventricular diameter, diameter of left ventricular posterior wall, end Systolic and diastolic measurement of left ventricle and ejection fraction were also calculated with M-mode. Pulsed Doppler was used to determine blood velocity and pre ejection period Ejection time, peak A velocity, Peak E velocity and Ejection time and then E/A, PEP/ET were calculated. Before blood samplings, patients were asked to rest for 15 minutes and then 5 cc of blood sample was taken and centrifuged at 3000 rpm/min about 10 minutes. Then, provided samples were stored at minus 80c. This process performed for both case and control groups. Finally, calcitonin and BNP were measured by ELISA kit (USA). To measure "a" the sample volume was positioned at the tips of the tricuspid and mitral valve leaflets in the apical four-chamber view which is defined as the time of interval between the end and the start of trans-mitral and trans-tricuspid flow. To measure "b", the sample volume

was placed to the left ventricular out flow tract just below the aortic valve in apical five-chamber view which is defined as the left ventricular ejection time. For the right ventricular outflow, velocity pattern was also recorded from the parasternal short-axis view with the Doppler sample volume positioned just distal to the pulmonary valve. MPI or the Tie Index was calculated as:  $a-b/b = IRT + ICT/ET$ . The left ventricular mass index (LVMI) was also calculated by equation of  $LVMI = 0.8 \cdot (1.04) (LVDD + PWT + IVSTD)^{**3} - (LVDD)^{**3} + 0.6 \cdot 8$ . Measures recorded in a specific sheet and entered to the SPSS 20 (SPSS, Inc., Chicago, IL) and analyzed based on proper statistical tests. Kolmogorov-Smirnov test was used for normality. If the distribution of data was normal, parametric tests and in contrast non-parametric were used. Mann-Whitney test, independent t-test, and Pearson correlation were the applied tests with significant level of  $< 0.05$ .

## Results

Sex distribution was 52.5% male and 47.5% females in case group and 50% male and 50% female in control. Weight, height and hemoglobin means were significantly higher in control ( $50.2 \pm 15.9$ ,  $158.47 \pm 15.47$  and  $14.32 \pm 1.09$ ) compared to case ( $38.21 \pm 8.82$ ,  $145.83 \pm 12.3$  and  $10.37 \pm 0.34$ ) group in the level of  $p < 0.001$  (Table 1). Table II shows the results of the test of normality for the principal variables. Test of K-S revealed that the majority of variables were distributed normally such as PEP/ET (K-S=1.041,  $p=0.229$ ), LVMI (K-S=795,  $p=0.553$ ) and LVDD (K-S=0.943,  $p=0.336$ ) and the minority of variables were non-normal such as BNP (K-S=4.952,  $p < 0.001$ ), and CGRP (K-S=, 1.660  $p=0.008$ ). Table III shows the results of independent t-test comparing case and control groups for those variables with normal distribution. The Table reports no statistically significant difference for echocardiographic findings such as

Ventricular posterior wall dimension in diastole ( $p=0.317$ ), Interventricular septal dimension in systole ( $p=0.536$ ), Ventricular posterior wall dimension in systole ( $p=0.058$ ), Peak A velocity ( $p=0.375$ ), and Peak E velocity in right ( $p=0.112$ ) when for the some findings such as PEP/ET in left ( $p<0.001$ ), ET in right ( $p<0.001$ ) and PEP/ET in right( $p<0.001$ ) the differences were significant. Table 4 reveals the results of non-parametric Mann Whitney U test comparing case and control groups for those variables with non-normal distribution. The result revealed that right MPI (Mean Rank= 41.675 for control and Mean Rank= 119.325 for patients with  $p<0.001$  ), left MPI (Mean Rank= 45.275 for control and Mean Rank= 115.725 for patients with  $p<0.001$ ), PEP in left (Mean Rank= 65.7 for control and Mean Rank= 95.3 for patients with  $p<0.001$ ) and ET in left (Mean Rank= 96.03 for control and

Mean Rank= 64.97 for patients with  $p<0.001$ ) had different significant mean rank in case and controls. Meanwhile, some of Echocardiographic findings demonstrated no differences in case and controls such as Interventricular septal dimension in diastole (Mean Rank= 78.36 for control and Mean Rank= 82.64 for patients with  $p=0.556$ ) and right DT (Mean Rank= 86.01 for control and Mean Rank= 74.99 for patients with  $p=0.130$ ). Table 5 depicts the correlation of BNP and CGRP as biomarkers with right and left echocardiography findings in both groups and all population in the study. In case group, the findings showed that BNP had a significant correlation with right MPI ( $r=0.229$ ,  $p=0.041$ ) and CGRP had significant correlations with PEP in right ( $r=0.238$ ,  $p=0.035$ ), PEP/ET in right ( $r=0.316$ ,  $p=0.005$ ), and peak A velocity in left( $r=0.245$ ,  $p=0.030$ ).

*Table I: Anthropometric and Hemoglobin mean in case and control*

| Variable                | Mean ±sd<br>(participants) | Mean ±SD<br>(patients) | Mean ±SD<br>(control) | P value( for case<br>and controls) |
|-------------------------|----------------------------|------------------------|-----------------------|------------------------------------|
| <b>Age(year)</b>        | 17.581±5.344               | 18.21 ±5.14            | 16.95 ±5.49           | 0.136                              |
| <b>Weight(kg)</b>       | 44.206±14.161              | 38.21 ±8.82            | 50.2 ±15.9            | 0.0001                             |
| <b>Height(cm)</b>       | 152.156±15.313             | 145.83 ±12.3           | 158.47 ± 15.47        | 0.0001                             |
| <b>hemoglobin(g/dl)</b> | 12.352 ±2.138              | 10.37 ±0.34            | 14.32 ±1.09           | 0.0001                             |

Table II: Results of Kolmogorov-Smirnov test for normality

| parameters | Mean    | sd     | K-S   | p     | parameters | Mean    | sd     | K-S   | p     |
|------------|---------|--------|-------|-------|------------|---------|--------|-------|-------|
| PEP/ET (L) | 0.34    | 0.051  | 1.041 | 0.229 | ICT (R)    | 38.369  | 21.059 | 0.877 | 0.426 |
| ET (R)     | 261.719 | 23.189 | 1.264 | 0.082 | Peak E (R) | 59.5    | 13.511 | 1.05  | 0.22  |
| PEP/ET (R) | 0.34    | 0.051  | 1.053 | 0.218 | Peak A (R) | 44.206  | 10.172 | 1.346 | 0.053 |
| PWD (L)    | 4.672   | 0.809  | 1.318 | 0.062 | MPI (R)    | 0.553   | 0.17   | 1.858 | 0.002 |
| IVSS (L)   | 11.196  | 1.742  | 1.041 | 0.229 | MPI (L)    | 0.517   | 0.13   | 1.782 | 0.003 |
| PWS (L)    | 5.444   | 0.872  | 1.212 | 0.106 | PEP (L)    | 89.644  | 10.347 | 2.107 | 0.000 |
| LVDD (L)   | 47.654  | 5.251  | 0.943 | 0.336 | ET (L)     | 263.294 | 23.736 | 1.323 | 0.06  |
| LVEDV (L)  | 69.481  | 20.585 | 0.594 | 0.873 | PEP R      | 89.306  | 10.696 | 2.446 | 0.000 |
| LVMI (L)   | 75.212  | 22.499 | 0.795 | 0.553 | IVSD (L)   | 8.195   | 1.396  | 1.533 | 0.018 |
| EF (L)     | 68.45   | 6.056  | 0.953 | 0.324 | LVDS (L)   | 29.501  | 4.659  | 1.516 | 0.02  |
| FS (L)     | 38.444  | 5.758  | 1.015 | 0.254 | IRT (L)    | 103.481 | 18.512 | 1.497 | 0.023 |
| LA         | 26.788  | 4.258  | 0.954 | 0.322 | AT (L)     | 60.506  | 13.036 | 2.706 | 0.000 |
| Aorta      | 24.559  | 3.463  | 1.091 | 0.185 | IRT (R)    | 113.613 | 23.67  | 1.392 | 0.042 |
| LA/ Aorta  | 1.096   | 0.173  | 1.317 | 0.062 | AT (R)     | 64.631  | 16.168 | 2.593 | 0.000 |
| ICT (L)    | 33.556  | 21.462 | 1.156 | 0.138 | DT (R)     | 141.606 | 20.987 | 1.717 | 0.005 |
| DT (L)     | 143.863 | 22.096 | 1.163 | 0.133 | E/A (R)    | 1.372   | 0.307  | 1.432 | 0.033 |
| Peak E (L) | 91.781  | 17.412 | 0.929 | 0.354 | BNP        | 6.309   | 18.918 | 4.952 | 0.000 |
| Peak A (L) | 50.913  | 11.712 | 1.084 | 0.191 | CGRP       | 3.784   | 2.906  | 1.66  | 0.008 |
| E/A (L)    | 1.863   | 0.461  | 0.96  | 0.316 | age        | 17.581  | 5.344  | 0.925 | 0.36  |

L= left heart , R = Right heart, PEP = Pre-ejection Period (m/s), ET = Ejection Time (m/s), PWD=Posterior wall dimension in diastole (mm), IVSS=Interventricular septal dimension in systole (mm), PWS=Posterior wall dimension in systole (mm), IVSD= Interventricular septal dimension in diastole (mm), LVDD=Left ventricular dimension diameter in diastole (mm), LVDS=Left ventricular dimension diameter in systole (mm), LVEDV= Left ventricular end diastolic volume(mm), LVMI= Left ventricular mass index (mm), EF= Ejection Fraction , FS= Fractional Shortening, LA=Left atrium, ICT= Isovolemic contraction time (m/s), DT = Deceleration time(m/s), MPI = Myocardial performance index, AT= Acceleration time (m/s), IRT= Isovolemic relaxation time, E/A =peak E/peak A velocity, BNP= Brain Natriuretic Peptides (pg/ml), CGRP= Calcitonin gene-related ptide( $\mu\text{g/l}$ ).

Table III : Results of independent t- test for normal variables in comparison in case and control groups

| variables for the left Heart                     | group   | Mean    | SD     | t      | P      |
|--|---------|---------|--------|--------|--------|
| Pre-ejection period                              | control | 0.316   | 0.038  | -6.896 | <0.001 |
|  | case    | 0.365   | 0.051  |        |        |
| Pre-ejection period/ejection time                | control | 0.316   | 0.039  | -6.792 | <0.001 |
|  | case    | 0.364   | 0.05   |        |        |
| Ventricular posterior wall dimension in diastole | control | 4.736   | 0.869  | 1.005  | 0.317  |
|  | case    | 4.608   | 0.744  |        |        |
| Interventricular septal dimension in systole     | control | 11.11   | 1.645  | -0.62  | 0.536  |
|  | case    | 11.281  | 1.841  |        |        |
| Ventricular posterior wall dimension in systole  | control | 5.575   | 0.839  | 1.909  | 0.058  |
|  | case    | 5.314   | 0.891  |        |        |
| Ventricular end-diastolic dimension              | control | 46.834  | 5.479  | -1.994 | 0.048  |
|  | case    | 48.474  | 4.911  |        |        |
| Ventricular end-diastolic volume                 | control | 63.32   | 18.166 | -3.957 | <0.001 |
|  | case    | 75.642  | 21.118 |        |        |
| Left ventricular mass index                      | control | 66.067  | 17.058 | -5.613 | <0.001 |
|  | case    | 84.356  | 23.633 |        |        |
| Ejection Fraction                                | control | 69.163  | 5.995  | 1.494  | 0.137  |
|  | case    | 67.738  | 6.071  |        |        |
| Fractional shortening                            | control | 38.813  | 5.222  | 0.809  | 0.42   |
|  | case    | 38.075  | 6.26   |        |        |
| Left atrium                                      | control | 25.268  | 3.795  | -4.82  | <0.001 |
|  | case    | 28.308  | 4.173  |        |        |
| Aorta  | control | 24.928  | 3.759  | 1.348  | 0.18   |
|  | case    | 24.191  | 3.119  |        |        |
| Left atrium / Aorta                              | control | 1.02    | 0.125  | -6.27  | <0.001 |
|  | case    | 1.173   | 0.18   |        |        |
| Isovolumic contraction time                      | control | 23.338  | 16.285 | -6.834 | <0.001 |
|  | case    | 43.775  | 21.221 |        |        |
| Deceleration Time                                | control | 149.813 | 23.675 | 3.527  | 0.001  |
|  | case    | 137.913 | 18.719 |        |        |
| Peak E velocity                                  | control | 88.95   | 16.894 | -2.078 | 0.039  |
|  | case    | 94.613  | 17.565 |        |        |
| Peak A velocity                                  | control | 51.738  | 12.921 | 0.89   | 0.375  |
|  | case    | 50.088  | 10.38  |        |        |
| E/A velocity ratio                               | control | 1.775   | 0.405  | -2.463 | 0.015  |
|  | case    | 1.951   | 0.498  |        |        |
|  | control |         |        |        |        |
| variables for the right heart                    | case    | Mean    | SD     | t      | P      |

|                                    |         |         |        |        |                  |
|------------------------------------|---------|---------|--------|--------|------------------|
| <b>Isovolumic contraction time</b> | control | 31.3    | 17.993 | -4.495 | <b>&lt;0.001</b> |
|                                    | case    | 45.438  | 21.625 |        |                  |
| <b>Peak E velocity</b>             | control | 57.8    | 12.928 | -1.599 | 0.112            |
|                                    | case    | 61.2    | 13.943 |        |                  |
| <b>Peak A velocity</b>             | control | 42.513  | 9.317  | -2.129 | <b>0.035</b>     |
|                                    | case    | 45.9    | 10.753 |        |                  |
| <b>Ejection time</b>               | control | 268.238 | 21.269 | 3.695  | <b>&lt;0.001</b> |
|                                    | case    | 255.2   | 23.321 |        |                  |

Table IV: Results of non-parametric Mann-Whitney U test for non-normal variable

| Variables for the left heart                         | groups  | Mean Rank | Sum of Ranks | Mann-Whitney U | p                |
|--|---------|-----------|--------------|----------------|------------------|
| <b>Myocardial performance index</b>                  | control | 45.275    | 3622         | 382            | <b>&lt;0.001</b> |
|  | case    | 115.725   | 9258         |                |                  |
| <b>Pre-ejection period</b>                           | control | 65.7      | 5256         | 2016           | <b>&lt;0.001</b> |
|  | case    | 95.3      | 7624         |                |                  |
| <b>Ejection time</b>                                 | control | 96.03125  | 7682.5       | 1957.5         | <b>&lt;0.001</b> |
|  | case    | 64.96875  | 5197.5       |                |                  |
| <b>Interventricular septal dimension in diastole</b> | control | 78.35625  | 6268.5       | 3028.5         | 0.556            |
|  | case    | 82.64375  | 6611.5       |                |                  |
| <b>Interventricular septal dimension in systole</b>  | control | 73.1375   | 5851         | 2611           | <b>0.044</b>     |
|  | case    | 87.8625   | 7029         |                |                  |
| <b>Isovolumic relaxation time</b>                    | control | 59.3875   | 4751         | 1511           | <b>&lt;0.001</b> |
|  | case    | 101.6125  | 8129         |                |                  |
| Variables for the right heart and biomarkers         | groups  | Mean Rank | Sum of Ranks | Mann-Whitney U | p                |
| <b>Myocardial performance index</b>                  | control | 41.675    | 3334         | 94             | <b>&lt;0.001</b> |
|  | case    | 119.325   | 9546         |                |                  |
| <b>Pre-ejection period</b>                           | control | 63.9      | 5112         | 1872           | <b>&lt;0.001</b> |
|  | case    | 97.1      | 7768         |                |                  |
| <b>Isovolumic contraction time</b>                   | control | 50.7125   | 4057         | 817            | <b>&lt;0.001</b> |
|  | case    | 110.2875  | 8823         |                |                  |
| <b>Acceleration time</b>                             | control | 74.44375  | 5955.5       | 2715.5         | 0.091            |
|  | case    | 86.55625  | 6924.5       |                |                  |
| <b>Deceleration time</b>                             | control | 86.00625  | 6880.5       | 2759.5         | 0.130            |
|  | case    | 74.99375  | 5999.5       |                |                  |
| <b>Peak E/A velocity ratio</b>                       | control | 83.01875  | 6641.5       | 2998.5         | 0.492            |
|  | case    | 77.98125  | 6238.5       |                |                  |
| <b>Brain Natriuretic Peptide</b>                     | control | 73.1125   | 5849         | 2609           | <b>0.043</b>     |
|  | case    | 87.8875   | 7031         |                |                  |
| <b>Calcitonin gene-related peptide</b>               | control | 57.5      | 4600         | 1360           | <b>&lt;0.001</b> |
|  | case    | 102.7848  | 8120         |                |                  |

*Table V: Results of biomarkers correlation with echocardiography findings in Patients*

| Right echo findings | statistics | Case group ( Patients) |        | Left Echo findings | Case group( Patients) |        |
|---------------------|------------|------------------------|--------|--------------------|-----------------------|--------|
|                     |            | BNP                    | CG RP  |                    | BNP                   | CGRP   |
| ICT                 | PC         | 0.200                  | 0.085  | MPI                | 0.212                 | 0.087  |
|                     | p          | 0.076                  | 0.456  |                    | 0.059                 | 0.445  |
| IRT                 | PC         | -0.052                 | -0.114 | PEP                | -0.065                | 0.068  |
|                     | p          | 0.650                  | 0.315  |                    | 0.566                 | 0.553  |
| AT                  | PC         | -0.059                 | -0.001 | ET                 | -0.187                | -0.097 |
|                     | p          | 0.602                  | 0.991  |                    | 0.097                 | 0.396  |
| PEP/ET              | PC         | -0.022                 | 0.316  | PEP/ET             | 0.071                 | 0.115  |
|                     | p          | 0.848                  | 0.005  |                    | 0.534                 | 0.313  |
| DT                  | PC         | 0.152                  | 0.184  | IVSD               | -0.022                | 0.007  |
|                     | p          | 0.179                  | 0.104  |                    | 0.847                 | 0.954  |
| E                   | PC         | -0.024                 | 0.106  | PWD                | 0.012                 | -0.017 |
|                     | p          | 0.830                  | 0.351  |                    | 0.913                 | 0.879  |
| A                   | PC         | 0.023                  | 0.014  | IVSS               | -0.025                | 0.067  |
|                     | p          | 0.842                  | 0.902  |                    | 0.828                 | 0.556  |
| A/E                 | PC         | -0.041                 | 0.134  | PWS                | 0.034                 | 0.020  |
|                     | p          | 0.721                  | 0.241  |                    | 0.764                 | 0.864  |
| MPI                 | PC         | 0.229                  | 0.052  | LVDD               | 0.104                 | 0.072  |
|                     | p          | 0.041                  | 0.648  |                    | 0.360                 | 0.530  |
| PEP                 | PC         | -0.040                 | 0.238  | LVDS               | 0.072                 | 0.032  |
|                     | p          | 0.724                  | 0.035  |                    | 0.528                 | 0.780  |
| ET                  | PC         | -0.030                 | -0.153 | LVDV               | 0.099                 | 0.098  |
|                     | p          | 0.795                  | 0.178  |                    | 0.385                 | 0.392  |
| Left Echo findings  | PC         | Case group( Patients)  |        | LVMI               | 0.013                 | -0.010 |
|                     | p          | bnp                    | CG RP  |                    | 0.910                 | 0.933  |
| AT                  | PC         | 0.010                  | 0.126  | FS                 | 0.035                 | 0.002  |
|                     | p          | 0.932                  | 0.270  |                    | 0.759                 | 0.987  |
| EF                  | PC         | 0.058                  | 0.041  | LA                 | 0.048                 | 0.165  |
|                     | p          | 0.613                  | 0.720  |                    | 0.673                 | 0.147  |



|            |    |        |      |        |        |       |
|------------|----|--------|------|--------|--------|-------|
| <b>DT</b>  | PC | -0.078 | 0.10 | Aorta  | 0.110  | 0.029 |
|            | p  | 0.495  | 0.35 |        | 0.331  | 0.801 |
| <b>E</b>   | PC | -0.075 | 0.01 | LA/ Ao | -0.046 | 0.118 |
|            | p  | 0.510  | 0.92 |        | 0.683  | 0.303 |
| <b>A</b>   | PC | -0.039 | 0.24 | ICT    | 0.116  | 0.080 |
|            | p  | 0.729  | 0.03 |        | 0.305  | 0.486 |
| <b>E/A</b> | PC | -0.040 | -    | IRT    | 0.039  | 0.020 |
|            | p  | 0.725  | 0.14 |        | 0.733  | 0.863 |

PEP =Pre-ejection Period , ET =Ejection Time, PWD= Posterior wall dimension in diastole, IVSS= Interventricular septal dimension in systole , PWS= Posterior wall dimension in systole , IVSD == Interventricular septal dimension in diastole ,LVDD= Left ventricular dimension diameter in diastole , LVDS = Left ventricular dimension diameter in systole, LVEDV= Left ventricular end diastolic volume , LVMI= Left ventricular mass index ,EF= Ejection Fraction , FS= Fractional Shortening , LA=Left atrium , ICT= Isovolemic contraction time , DT = Deceleration time , MPI = Myocardial performance index , AT= Acceleration time , IRT= Isovolemic relaxation time , E/A =peak E/peak A velocity, BNP= Brain Natriuretic Peptides, CGRP= Calcitonin gene-related peptide.

## Discussion

The analysis of the present study showed that means of weight, height, and hemoglobin were higher in control compared to case. BNP and CGRP levels had higher mean ranks in case compared to controls. Accordance with correlation analysis, it was observed that the level of BNP had a significant correlation with right MPI in patients but CGRP had significant correlation with more echocardiographic findings such as PEP, PEP/ET in right heart and peak A velocity in left heart. Kremastinos reported that NT-Pro BNP was increased in major beta thalassemia patients compared to controls and this increase in patients was likely due to age and left ventricular diastolic dysfunction (11) but Whal held that BNP increasing was a reflection of preload volume and afterload pressure which release hormones in response to cardiomyocytes stretch (12 )because of choosing asymptomatic patients in the present study and comparing different biomarkers compared to Kremastinos and Whal studies, the results could be

comparable. Hamodraka observed that both BNP and NT Pro BNP would be increased in major beta thalassemia patients with systolic and diastolic dysfunction (13). In the present study, an increase of BNP in major beta thalassemia patients with diastolic and systolic dysfunction resulte was similar with the mentioned study regarding BNP. Aessopos and Meloni demonstrated that cardiac effects were the main causes of mortality in major beta thalassemia patients and BNP level increases in patients with global or local left ventricular diastolic dysfunction due to wall stretch in left ventricle (14,15). Direct correlation was found between MPI of right heart and BNP in patients. This result revealed that BNP was a good biomarker in early diagnosis of heart involvement in thalassemia patients. Tanner showed that myocardial sidrosis occurred in two third of thalassemia patients who were treated with deferoxamine and BNP was not a valuable biomarker to evaluate myocardial sidrosis (16) which somehow was dissimilar with

the present study results regarding myocardial dysfunction.

In Aessopos study had been explained that forecasted the risk of heart failure progress by BNP and it would be increased in patients with obvious cardiac dysfunction but did not show the severity of failure (17). Maisel study showed that Pro BNP was useful like BNP in diagnosis of heart failure with dyspnea and acute heart failure (18). In the present study, patients were asymptomatic which was different with Aessopos and Maisel researchers that had been performed on patients with heart failure. Therefore, with respect to this different, it was found that BNP and Pro BNP were useful tools to diagnosis of cardiac involvement in patients with major thalassemia. Xin conducted a study on the relationship between echocardiographic findings and CGRP of right heart and observed that CGRP level had indirect correlation with ventricular MPI and pulmonary hypertension (19). There was no relationship between CGRP level and ventricular MPI in major thalassemia patients in the present study but a strong relationship with PEP, PEP/ET in right heart and Peak A velocity in left were observed. Marangoni were analyzed a few diabetic rats by injecting streptozotocin (STZ) to them. Then LVDD, LVSD, IVSD, IVSS, FS, E/A, IVRT, and DT were measured and observed that these echocardiographic findings were lower in controls compared to cases (20). The present study came to the conclusion that LVDD, E/A, IVRT were lower in control group similar to Marangoni study. Main differences between these two studies were related to the subjects and causes of cardiac involvement. Suman expressed that MPI was very high in chelated thalassemia children which can be used as an early biomarker to diagnose ventricular dysfunction (21). Along with Suman outcomes on chelated thalassemia children, a few reports revealed that the prognoses of patients with elevated BNP levels were consistently poorer in quality

and low active life expectancy in compared with patients in lower BNP levels. This consistency was due to the presence of LV diastolic dysfunction in thalassemia major (22, 23). Therefore, BNP levels were used to predict the prognosis of patients with cardiovascular disease, particularly in patients with LV diastolic dysfunction. Hsu measured CGRP levels in CHD patients and concluded that an increase of CGRP level would be associated with pulmonary hypertension (9). In CHD patients, a main causes of CHF (Congestive Heart Failure) were volume overload and volume pressure. In major thalassemia patients, cause of cardiac dysfunction was iron overload with regarde to the immunologic factors. In compared with the present study, Hsu did measured CGRP on CHD patients whereas in this study CGRP was measured in asymptomatic patients with major thalassemia. 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, Anand's 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 (24). The difference between Anand's study and the present was related to the samples under investigation (animal models instead of humans). A study on the impact of CGRP on heart contractility and hemodynamic in idiopathic dilated cardiomyopathy patients revealed that after CGRP injection, norepinephrine increased but blood pressure decreased. Hence, CGRP can be recommended for the treatment of heart failure (25). Noori performed a study on dilated cardiomyopathy patients and showed that severity of illness based on the Ross classification had positive correlation with BNP and BNP had a significant correlation with majority of echocardiographic parameters (8). With respect to this fact that the majority of

thalassemia patients suffer from type of cardiomyopathy and the association between these two diseases, the results of the present study would be in line with Noori's study.

### Conclusion

The majority of echocardiography findings were increased in the thalassemia patients compared to controls. Biomarkers of CGRP and BNP were increased in patients with major thalassemia compared to controls. These biomarkers had significant correlation with some of echocardiography findings such as MPI, PEP/ET, and PEP in right heart and Peak A velocity for the left heart. Hence, it seemed that CGRP and BNP can be useful in early diagnosis of cardiac involvement in patients with major thalassemia. In addition, it was found that BNP can be a stronger biomarker than CGRP.

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### Conflict of interest

The authors would like to declare no conflict of interests.

### References

- 1.Noori MN, 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  $\beta$ -Thalassemia Major. *Iran Red Crescent Med J*. 2014; 16(5).
- 2.Noor Mohammad Noori, 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.
- 3.Noori N, 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. *The Journal of Tehran University Heart Center*. 2013 Jan;8(1):35.

4.Noori N, Mahjoubifard M, Alavi SM, Hosseini S, Sanati H, Mirmesdagh Y. Diagnostic value of electrocardiography compared with echocardiography in measuring left ventricular mass index in major thalassemia patients over 10 years of age. *The Journal of Tehran University Heart Center*. 2013 Jul;8(3):121.

5.Gessler P, Knirsch W, Schnitt B, et al : Prognostic Value of Plasma N-Terminal Pro-Brain Natriuretic Peptide in Children with Congenital Heart defects and open-heart Surgery, *J Pediatric* 2006;148:372-6.

6.Law YM, Keller BB, Feingod BM, et al: Usefulness of Plasma B-type Natriuretic Peptide to Identify Ventricular Dysfunction in Pediatric and Adult Patients With Congenital Heart Disease, *Am J Cardiol* 2005;95:474-478.

7.Kremastinos DTH, Tsiapras DP, Kostopoulou AG, et al : NT-Pro BNP Level and Diastolic Dysfunction in Beta Thalassemia Major, *European Journal of Heart Failure* 2007;9:531-536.

8.Noori NM, Mahjoubifard M, Shahramian I, Teimouri A, Jahangirifard A. Comparison between Procalcitonin, Brain Natriuretic Peptide, and Uric Acid in Children with Cardiomyopathy and Controls. *BioMed research international*. 2015 Oct 1;2015.

9.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: 867-875.

10.Zhang ZW, Shi SS, Li JH, Lin R, Hu J, Wang X :Levels of plasma endothelin and calcitonin gene-related peptide in children with congenital heart disease and pulmonary hypertension, *World J Pediatr* 2006;2:139-142.

11.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  $\beta$ -thalassemia major. *American Heart Journal* 2010; 159(1): 68-74.
12. Wahl S, Vichinsky E. Pulmonary hypertension in hemolytic anemias, *f1000 Medicine Reports* 2010, 2:10.
13. Hamodraka E, Paraskevaidis I, Parissis J, Tsiapras D, Kostopoulou A, Vrettou E, Kapsali E, Kremastinos D. NT-pro BNP is a better biomarker than BNP for the identification of left ventricular dysfunction in beta-thalassemia major patients *Eur J Heart Fail Suppl* 2008;7(Suppl 1): 192-193.
14. Aessopos A, Farmakis D, Polonifi A, Tsironi M, Fragodimitri C, Hatziliami A, Karagiorga M, Diamanti-Kandarakis E. Plasma B-type natriuretic peptide concentration in  $\beta$ -thalassaemia patients, *European Journal of Heart Failure* 2007; 9: 537-541.
15. Meloni A, Pepe A, Zyw L, Positano V, Chiara Dell'Amico M, Passino C, et al. N-terminal fragment of proBNP is a marker of risk for right ventricular dysfunction and cardiac complications in thalassemia major, *Journal of Cardiovascular Magnetic Resonance* 2010, 12 (Suppl 1): 284.
16. Tanner A, Galanello R, Dessi C, Westwood MA, Smith GC, Nair SV, et al. Myocardial Iron Loading in Patients with Thalassemia Major on Deferoxamine Chelation, *Journal of Cardiovascular Magnetic Resonance* 2006; 8(3): 543-547.
17. A Aessopos, V Berdoukas, M Tsironi. The brain natriuretic peptide in diagnosis and treatment of heart failure. *E heart in transfusion dependent homozygous thalassaemia today – prediction, prevention and management*, *European Journal of Haematology* 2008; 80 (2) : 93-106.
18. A Maisel, C Mueller, R Nowak, W F Peacock, JW Landsberg, P Ponikowski, et al: Mid-Region Pro-Hormone Markers for Diagnosis and Prognosis in Acute Dyspnea: Results From the BACH (Biomarkers in Acute Heart Failure) Trial, *Journal of the American College of Cardiology* 2010; 55( 19): 2062-2076.
19. XIN Pei-qian, SUN Ming-bo, SUN Guo-hua, DING Hong, ZHU Wei-dong, WU Zhen-yu, QIAO Shan-hai. 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* .2005-01-001.
20. 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.
21. Suman RL, Sanadhya A, Singh J, Meena P. Myocardial performance index in children with  $\beta$ -thalassemia major. *Indian Journal of Child Health*. 2016 Aug 15;3(3):212-5.
22. Gong H, Wang X, Shi YJ, Shang WJ, Ling Y, Pan LJ, Shi HM. Correlation between brain natriuretic peptide levels and the prognosis of patients with left ventricular diastolic dysfunction. *Experimental and Therapeutic Medicine*. 2016 Jun 1;11(6):2583-9.
23. Fathy WM, El-Aziz WF, Ragab SM, Helal RT. Plasma brain natriuretic peptide concentration in  $\beta$ -thalassemia patients. *Menoufia Medical Journal*. 2015 Oct 1;28(4):978.
24. Anand IS, Gurden J, Wander GS, O'Gara P, Harding SE, Ferrari R, et al. Cardiovascular and hormonal effects of calcitonin gene-related peptide in congestive heart failure. *J Am Coll Cardiol*. 1991;17(1):208-17.
25. Dubois-Randé JL, Merlet P, Benvenuti C, Sediame S, Macquin-Mavier I, Chabrier E, Braquet P, Castaigne A, adnots. Effects of calcitonin gene-related peptide on cardiac contractility, coronary hemodynamics and myocardial energetics in idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1992;70(9):906-12.

