http:// ijp.mums.ac.ir
Original Article (Pages: 6077-6094)

Diagnostic Value of NT-pro BNP Biomarker and Echocardiography in Cardiac Involvements in Beta-thalassemia Patients

Noor Mohammad Noori[1], *Alireza Teimouri[1], Maryam Nakhaey Moghaddam[1]

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

Abstract

Background
N-terminal pro–B-type natriuretic peptide (NT-proBNP) is a marker to evaluate the cardiac involvement in thalassemia. We aimed to evaluate conventional and tissue Doppler echocardiography findings and its relation to plasma NT-pro BNP, Ferritin and Iron levels in beta-thalassemia patients.

Materials and Methods
This study performed on 164 participants equal of major beta- thalassemia patients (n=82), and controls (82 healthy children with normal cardiovascular status). The patients collected from outpatients after applied exclusion criteria. Blood samples were taken from participants in fasting to measure NT- pro BNP, Ferritin and Iron serum. Participants were under echocardiography by Pediatric cardiologist. The level of error considered as 0.05 for data analysis by SPSS version 20.0.

Results
NT- pro BNP, Iron, Ferritin, left S', left A', right A', EF, FS, left and right A/A'; right and left MPI were significantly different in patients group (P<0.05). FS and EF were higher in younger's and left and right MPI were lower. In higher level of NT-pro BNP right S' had converse trends compared with the Iron, its level was higher in patients > 10 years, and FS had different values significantly in lower levels. In lower level of left E/E' resulted that FS was significantly higher in the age group < 10 years. Right peak E velocity was significantly higher in younger's in higher level of left E/E'. Right E/E' was significantly higher in elders in lower level of left E/E' (P<0.05).

Conclusion
The study confirmed that NT-pro BNP increases in thalassemia and association with age and LV diastolic dysfunction. NT-pro BNP with E/E' and S' were shown diastolic and systolic dysfunction in thalassemia. Therefore, an increased level of ferritin and NT-pro BNP can be used as a marker for the intensification of iron chelation therapy, which reverses iron-induced cardiomyopathy.

Key Words: Children, Cardiac involvement, Echocardiography, NT-pro BNP, Beta-thalassemia.

*Please cite this article as: Noori NM, Teimouri A, Nakhaey Moghaddam M. Diagnostic Value of NT-pro BNP Biomarker and Echocardiography in Cardiac Involvements in Beta-thalassemia Patients. Int J Pediatr 2017; 5(11): 6077-94. DOI: **10.22038/ijp.2017.24951.2110**

Corresponding Author:

Dr. Alireza Teimouri, Assistant Professor, Children and Adolescents Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

Email: alirezateimouri260@ gmail.com

Received date: Jul.27, 2017; Accepted date: Aug. 22, 2017

1- INTRODUCTION

Thalassemia is an autosomal recessive hemoglobinopathy characterized by chronic hemolytic anemia with the frequency of 60,000 to 100,000 in newborns around the world (1). It is endemic in Mediterranean and in several Asian countries, migration has spread it throughout the world and in Iran, Sistan and Baluchestan province is one of the provinces with high prevalence of thalassemia (1, 2). In patient with thalassemia, regular blood transfusion programs and chelation treatment have considerably improved the patients' survival (3). However, a consequence of chronic transfusion therapy is secondary iron overload, which adversely effect on heart functions, liver and other organs, the cardiac complications are still the primary leading cause of death in beta thalassemia for young adults (4). Cardiac dysfunctions have traditionally been attributed to iron-overload related to repeated transfusions in thalassemia and increased intestinal absorption rate combined with a sustained state of increased cardiac output (5).

The most common causes of death in thalassemia patients are congestive heart failure, secondary to iron overload and high-output state (2). Since early diagnosis of beta thalassemia may lead to intensive chelating treatment and consequently allow a better life and recently several conventional echocardiography parameters have been investigated to assess cardiac functions in asymptomatic thalassemia patients (6). Heart failure onset remains poorly predictable, because echocardiography markers remain normal until heart failure is clinically manifest. It has been demonstrated that, unlike Ejection fraction (EF), long axis function is relatively independent from chronic volume overload and could be a sensitive marker of early myocardial dysfunction (7). In comparison, N-terminal pro–B-type natriuretic peptide (NT-proBNP) is more stable and has a longer half-life about 2 hours in compared to Brain Natriuretic Peptide (8). Increasing levels of NT-pro BNP in thalassemia patients with left ventricular diastolic dysfunction is directly related to age and it is a good biomarker for early diagnosis of left ventricular dysfunction. In the early phases of the cardiac involvement the level of NT-pro BNP increases before an increase in diastolic pressure and there is a strong relationship between plasma levels of NT-pro BNP and iron overload (9).

A significant relationship between NT-pro BNP and some diastolic dysfunctions has been observed (10). It is approved that an increase level of NT-pro BNP can be used as a tool for primary detection of cardiac hemosiderosis, and is confirmed iron chelation therapy which may reverse iron-induced cardiomyopathy. Patients with major beta- thalassemia faced to an increase in the level of NT- pro BNP, when diastolic left ventricular dysfunction increased. High serum of NT- pro BNP is related to levels of E / E' in Doppler tissue imaging (DTI) in these patients (8-10).

Tissue Doppler Imaging (TDI) is a relatively new Doppler ultrasound modality that records regional systolic and diastolic velocities within the myocardium. It allows quantitative measurement of both systolic and diastolic velocities directly from the ventricular myocardium with the determination of the extent of mitral annular displacement in systole and diastole (11). This new technique can show additional information compared with other echocardiography techniques, detecting even minor changes before the occurrence of abnormal indices of global ventricular dysfunction (2). Recent studies have revealed a significant relationship between elevated NT-pro BNP levels and diastolic dysfunction in these patients with preserved systolic function (2, 3). Thalassemia patients may remain asymptomatic and global left ventricular (LV) function may be preserved until late in the disease process. Therefore, early detection of myocardial dysfunction may be useful in the management plans. In order to clarify these issues, the present study was performed to evaluate conventional and tissue Doppler echocardiography findings and its relation to plasma NT-pro BNP, Ferritin and Iron levels in thalassemia patients in two pediatric centers of cardiac and specific diseases in Ali Asghar hospital, Zahedan city, Sistan and Baluchestan province, Iran, from August 2015 to June 2016.

2- MATERIALS AND METHODS

2-1. Study Design and Population

This case-control study was performed on 82 patients with major beta-thalassemia aged 5 to 18 years were diagnosed based on hemoglobin electrophoresis and enrolled for the study. Patients were selected from those attending outpatient clinics and inpatient wards in Ali Ibn Abi Talib hospital's pediatric clinics in Zahedan city, Sistan and baluchestan province, Iran. The control group included of 82 matched in age and gender healthy individuals with normal cardiovascular status from those who attended to the hospital for routine checkup. Sample size was based on the cost, ethical limitation especially for controls and age consideration. The following formula applied to calculate sample size.

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(difference)^2}$$

Where: n= Sample size, r = ratio of controls to cases = 1, σ=Standard deviation of the outcome variable=1.6, effect size (the difference in means) = 0.7, $Z\beta$= Represents the desired power =typically.84 for 80% power and $Z_{\alpha/2}$= Represents the desired level of statistical significance = typically = 1.96 for 95% of confidence interval (14).

2-2. Inclusion and exclusion criteria

Patients who included to the study were diagnosed based on hemoglobin electrophoresis and selected randomly from clinical outpatients and inpatients. The control group included of matched in age and gender healthy individuals attended to the hospital for routine checkup. Exclusion criteria were rheumatic heart disease, history of smoking, hypertension, present or past history of overt heart failure also excluded from the study with other causes of heart failure, other than iron overload such as primary hemosiderosis, hypercalcemia, thyrotoxicosis, obvious valvar disease, rhythm abnormality, structural, active infection, other systemic inflammatory diseases, and renal insufficiency.

2-3. Measures

2-3.1. NT-pro BNP, ferritin and iron Measurements

Three milliliters (ml) of blood was taken from all patients at 8.00 AM after an overnight fasting. The sample was centrifuged at 3,000 rpm/min for 10 minutes at 5 °C. Separated serum of NT-pro BNP, ferritin and iron were kept in -70cc refrigerator until measuring. The samples transferred to the biochemistry library of Zahedan University of Medical Sciences with considering the cold chain circumstances and then 250 microns isolated serum samples selected for measuring the levels of NT-pro BNP by ELISA kit, and same serum samples selected for measuring the levels of ferritin and iron levels by using ELISA kit (USA).

2-3.2. Echocardiography findings

Major proceedings were performed on both patients and controls such as medical history, physical examination; chest X-ray and electrocardiogram (ECG) participants without breath holding and view was taken in the mitral valve surface in parasternal position. The whole study population went under conventional echocardiography and Doppler tissue echocardiography, using My Lab 60 instrument with 3-8-MHz transducers (made in Italy). Parameters namely ejection fraction (EF), fractional shortening (FS), the velocity of the blood flow through the heart valves, as well as peak A velocity (A), peak E velocity (E), and myocardial performance index (MPI) were measured by M mode. The sample volume was positioned at the tips of the tricuspid and mitral valve leaflets in the apical four-chamber view to enable the measurement of (a): which is the time of interval between the end and the start of trans-mitral and trans-tricuspid flow.

The sample volume was thereafter relocated to the left ventricular outflow tract just below the aortic valve (apical five-chamber view) so as to measure (b): which is the left ventricular ejection time. 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 for the measurement of (b).

MPI calculated as: a-b/b = IRT + ICT/ET (12-14) (**Figure.1**). Doppler tissue echocardiography (DTE) was another method that was performed from the apical four-chamber view, and 3mm pulsed ultrasound Doppler samples before echocardiography. Echocardiography was performed 48-72 hours after packed red blood cell transfusion on patients (13), by same with using of my lab 60 with transducer 3-8 made in Italy.

But for the control group only echocardiography was performed. For having high precision echocardiography repeated 3 cycles in 2D, M-Mode, Doppler and tissue Doppler imaging methods and the average was considered. Echocardiogram applied in volume was placed at the level of lateral mitral annulus. Myocardial velocity profiles of the lateral tricuspid annulus and lateral mitral annulus were obtained by placing the sample volume at the junction of the tricuspid annulus and the right ventricle (RV) free wall and at the junction of the mitral annulus and LV posterior wall, respectively. With this modality, the recorded values were the early (E) and late (A) diastolic mitral and tricuspid annular velocities, and the ratio of E/A (13-15).

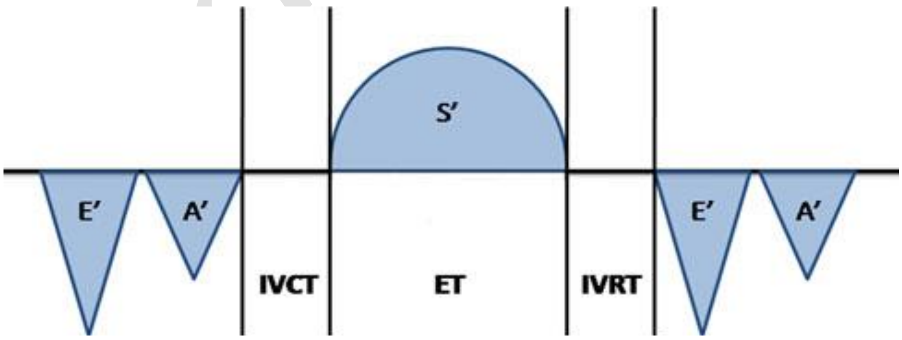

Fig1: Diagram of Doppler Tissue Echocardiography waves: S', systolic wave; E', early diastolic wave; A', late diastolic wave (14).

2-4. Ethical considerations

This study is a part of a thesis. The study was approved as a thesis for the degree of pediatric specialty by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (ID number: 6798). The written informed consent was obtained from all participants

2-5. Data analysis

All statistical procedures were performed using SPSS (Statistical Package for Social Sciences) Windows version 18.0 (SPSS Inc, Chicago, IL, USA). Data were summarized by mean ± standard deviation (SD). Kolmogorov-Smirnov test was performed for normality. Statistically significant differences of continuous variables were determined by using the independent samples t-test and Mann-Whitney U test based on normality distribution. A p-value < 0.05 was considered statistically significant.

3- RESULTS

This case-control study was performed to evaluate conventional and tissue Doppler echocardiography findings and its relation to plasma NT-pro BNP, Ferritin and Iron levels in thalassemia patients. The participants were collected from those who referred to Ali Ibn Abi Talib hospital's pediatric clinics in Zahedan city, Sistan and baluchestan province, Iran, assessed. From 164 participants, 82 were patients with major beta-thalassemia aged 5 to 18 years and same number were control which they matched in age (11.85±3.36 in control and 10.84±3.41 in patients) (p=0.099). Test of normality showed that almost all the variables had non normal distribution in the case of case and control participants. But some of variables such as height, weight, age, left A', EF, peak E in left, peak A in right, MPI left, and right had normal distribution in the case of only patients consideration (**Table.1**).

Non-parametric test of Mann Whitney showed that variables of NT- pro BNP (MW=443, p<0.001), Iron (MW=79.5, p<0.001), Ferritin (MW=101, p<0.001), left S' (MW=2715, p=0.033), left A' (MW=2635, p=0.017), right A' (MW=2594.5, p=0.012), EF(MW=1971, p<0.001), FS(MW=1479, p<0.001), left A/A' (MW=2350, p<0.001), right A/A' (MW=2714, p=0.033), right MPI (MW=2097.5, p<0.001), and left MPI (MW=1663, p<0.001), were significantly different between case and controls. Amongst the variables in the study, the others were non-significant (p > 0.005) (**Table.2**).

In the **Table.3** patients comparison based on parametric and Non-parametric test for two groups of age < 10 years and patients belong to age group of 10-18 years. The table showed that Mann Whitney test revealed the level of significant (MW=305, p<0.001) for the variable of FS. Next segment of the table based on the t-test showed that height (t=11.73, p<0.001), weight (t=10.72, p<0.001), age (t=8.5, p<0.001), EF (t=5.13 p<0.001), MPI left (t=4.23, p<0.001), and right (t=5.67, p<0.001), were significantly different.

Amongst the variables in the study, Iron, right S' and FS were different in age groups categorization, so that Iron were different significantly (MW=208.5, p=0.026) in the case of patients who had lower level of 847.5 for NT- pro BNP regarding to patients who had higher level. Right S' had convers trends compared with Iron so that its level were higher in patients who aged > 10 years regarding to the higher level of NT- pro BNP (MW=52.5, p=0.023).

FS had different values significantly in lower levels of NT- pro BNP (MW= 126, p<0.001), and higher level (MW=39, p=0.004) regarding age groups. For the normal distributed variables, anthropometric variables of height, weight and age were significantly different in patients with two levels of NT- pro BNP regarding age groups. Three echocardiography findings of EF (for NT- pro BNP <847.5: (t=3.87, p<0.001), and for NT- pro BNP > 847.5: (t=3.2, p=0.004), right MPI (for NT- pro BNP < 847.5: (t=-2.87, p=0.006), and for NT- pro BNP > 847.5: (t=-3.28, p=0.003), and left MPI (for NT- pro BNP <847.5: (t=-4, p<0.001), and for NT- pro BNP > 847.5: (t=4.15, p<0.001), had different levels in two age groups (**Table.4**).

Table.5 showed the results for variables values comparison between age groups in both left E/E' levels regarding the cutoff point 9.0. Results of the first part of the table revealed that variables of FS (MW=152.50, p<0.001) were significantly higher in the age group < 10 years for the levels of left E/E' lower than cutoff point. Right peak E velocity (MW=8.00, p=0.017) were significantly higher in the age group < 10 years for the levels of left E/E' more than cutoff point; and right E/E' (MW=351.50, p<0.022) were significantly higher in the age group > 10 years for the levels of left E/E' lower than cutoff point. Part two of the table showed the results due to independent t-test so that height, weight and age were significantly different between age groups. EF (for E/E' < 9: T=5.36, p<0.001), right MPI (for E/E '< 9: T=-4.95, p<0.001), and left MPI (for E/E' < 9: T=-5.92, p<0.001), had different values in age group <10 years compared to > 10 years aged.

Part one of the **Table.6** showed the results due to Mann Whitney test so that Iron and FS were significantly different between age groups. Iron (for S'<8: MW=46, p<0.028, higher in age group > 10 years), FS (for S'<8: MW=44, p=0.022, lower level in age group > 10 years, and for S'>8: MW=108.5, p<0.001, lower level in age group > 10 years). Part two of the **Table.6** showed that height, weight and age were different in age groups in both levels of left S'. Same trends observed for EF, Right MPI and left MPI. EF had significantly lower value in age group of > 10 years (for left S' < 8: t=2.64, p=0.013 and for left S' > 8: t=4.12, p<0.001). Right MPI had significantly higher value in age group of > 10 years (only for left S' > 8: t= -4.64, p<0.001) and left MPI had same trends (only for left S' > 8: t= -6.96, p<0.001).

Table-1: Test of normality for clinical and laboratory findings of β-thalassemia major patients and control group

Variables	All participant				Patients			
	Mean	SD	K.S	P-value	Mean	SD	K.S	P-value
NT- pro BNP	638.711	841.780	0.278	0.000	1073.746	1003.273	0.288	0.000
Iron	172.598	103.136	0.223	0.000	265.439	58.576	0.235	0.000
Ferritin	2402.427	3758.241	0.265	0.000	4757.378	4146.572	0.129	0.002
Height	135.561	18.286	0.059	0.200	133.305	17.590	0.063	0.200
Weight	31.396	11.577	0.103	0.000	29.976	10.628	0.095	0.066
Age	11.378	3.642	0.080	0.012	10.768	3.390	0.093	0.076
Left S'	8.593	1.989	0.150	0.000	8.160	2.131	0.155	0.000
Left E'	14.459	3.435	0.101	0.000	14.143	4.190	0.115	0.009
Left A'	6.331	1.869	0.099	0.000	5.969	1.997	0.097	0.052
Right S'	5.467	3.519	0.201	0.000	5. 9217	3. 9956	0.190	0.000

Right E'	14.343	3.307	0.138	0.000	13.969	4.140	0.141	0.000
Right A'	6.920	2.735	0.141	0.000	6.620	3.206	0.172	0.000
EF	74.439	10.776	0.161	0.000	72.463	7.682	0.095	0.063
FS	43.470	7.165	0.146	0.000	40.537	6.236	0.100	0.042
Left E	101.545	19.276	0.065	0.091	101.759	18.614	0.079	0.200
Left A	53.673	10.734	0.160	0.000	55.609	12.178	0.098	0.048
Right E	69.198	11.974	0.082	0.009	68.818	12.738	0.110	0.015
Right A	49.569	11.242	0.086	0.005	50.043	9.989	0.078	0.200
Left E/ E'	11.920	55.974	0.446	0.000	16.730	79.073	0.446	0.000
Right E/E'	7.897	34.508	0.441	0.000	10.912	48.743	0.438	0.000
Left A/A'	16.038	76.213	0.452	0.000	23.377	107.468	0.452	0.000
Right A/A'	13.372	57.632	0.445	0.000	19.001	81.141	0.438	0.000
Left MPI	0.685	0.181	0.077	0.019	0.745	0.210	0.073	0.200
Right MPI	0.600	0.226	0.133	0.000	0.704	0.265	0.083	0.200

NT-proBNP = N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A' = late diastolic velocity; S' = systolic peak velocity, EF = ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, E/E' = early diastolic pulsed-wave Doppler / early diastolic velocity, A/A' = late diastolic wave / late diastolic velocity MPI = myocardial performance index, SD= standard deviation, K.S= Kolmogorov–Smirnov test.

Table-2: Clinical and laboratory findings of β-thalassemia major patients and control group

Variables	Groups	Number	Mean	SD	Mean Rank	Sum of Mean Rank	Mann-Whitney U	P-value
NT-pro BNP	Control	82	203.68	190.24	46.90	3846.00	443	<0.001
	Case	82	1073.75	1003.27	118.10	9684.00		
Iron	Control	82	79.76	22.852	42.47	3482.50	79.5	<0.001
	Case	82	265.44	58.576	122.53	10047.50		
Ferritin	Control	82	47.48	23.099	42.73	3504.00	101	<0.001
	Case	82	4757.38	4146.572	122.27	10026.00		
Height	Control	82	137.82	18.792	87.20	7150.50	2976.5	0.205
	Case	82	133.30	17.590	77.80	6379.50		
Weight	Control	82	32.82	12.355	87.34	7161.50	2965.5	0.192
	Case	82	29.98	10.628	77.66	6368.50		
Age	Control	82	11.85	3.655	88.59	7264.00	2863	0.099
	Case	82	10.84	3.412	76.41	6266.00		
Left S'	Control	82	9.0256	1.74376	90.41	7414.00	2713	0.033
	Case	82	8.1596	2.13107	74.59	6116.00		
Left E'	Control	82	14.7756	2.44725	83.88	6878.00	3249	0.710
	Case	82	14.1430	4.18985	81.12	6652.00		
Left A'	Control	82	6.6939	1.66580	91.37	7492.00	2635	0.017
	Case	82	5.9689	1.99680	73.63	6038.00		
Right S'	Control	82	5.011	2.899	76.14	6243.50	2840.5	0.085
	Case	82	5.9217	3.996	88.86	7286.50		

Right E'	Control	82	14.7183	2.14102	83.41	6840.00	3287	0.805
	Case	82	13.9687	4.14045	81.59	6690.00		
Right A'	Control	82	7.2195	2.14317	91.86	7532.50	2594.5	0.012
	Case	82	6.6201	3.20599	73.14	5997.50		
EF	Control	82	76.4146	12.91277	99.46	8156.00	1971	<0.001
	Case	82	72.4634	7.68226	65.54	5374.00		
FS	Control	82	46.4024	6.85450	105.46	8648.00	1479	<0.001
	Case	82	40.5366	6.23648	59.54	4882.00		
Peak E (Left)	Control	82	101.3305	20.02905	82.24	6743.50	3340.5	0.944
	Case	82	101.7585	18.61411	82.76	6786.50		
Peak A (Left)	Control	82	51.7366	8.71640	75.95	6228.00	2825	0.077
	Case	82	55.6085	12.17826	89.05	7302.00		
Peak E (Right)	Control	82	69.5768	11.22439	84.41	6922.00	3205	0.605
	Case	82	68.8183	12.73833	80.59	6608.00		
Peak A (Right)	Control	82	49.0951	12.41443	77.54	6358.00	2955	0.181
	Case	82	50.0427	9.98851	87.46	7172.00		
Left E/E'	Control	82	7.1098	2.32039	81.41	6675.50	3272.5	0.768
	Case	82	16.7298	79.07318	83.59	6854.50		
Right E/E'	Control	82	4.8811	1.41049	81.40	6675.00	3272	0.767
	Case	82	10.9122	48.74279	83.60	6855.00		
Left A/A´	Control	82	8.6984	5.50273	70.16	5753.00	2350	0.001
	Case	82	23.3774	107.46791	94.84	7777.00		
Right A/A´	Control	82	7.7428	5.98050	74.60	6117.00	2714	0.033
	Case	82	19.0009	81.14134	90.40	7413.00		
Right MPI	Control	82	.6256	0.12084	67.08	5500.50	2097.5	<0.001
	Case	82	.7449	0.21035	97.92	8029.50		
Left MPI	Control	82	.4970	0.10304	61.78	5066.00	1663	<0.001
	Case	82	.7038	0.26480	103.22	8464.00		

NT-proBNP=N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A'= late diastolic velocity; S' =systolic peak velocity, EF= ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, MPI: myocardial performance index, E/E'= early diastolic pulsed-wave Doppler /early diastolic velocity, A/A'= late diastolic wave/ late diastolic velocity, Peak E= early mitral and tricuspid valve flow velocity , PeakA= late mitral and tricuspid valve flow velocity, SD= standard deviation.

Table-3: Age comparison of clinical and laboratory findings of β-thalassemia major patients

Variables	Age, year	Number	Mean	SD	Mann-Whitney U	P-value
NT-pro BNP	<10	33	1120.7009	1154.88	746.5	0.558
	10-18	49	1042.1239	898.25507		
Iron	<10	33	247.79	68.286	638.5	0.107
	10-18	49	277.33	48.198		
Ferritin	<10	33	4915.06	5180.814	729	0.452
	10-18	49	4651.18	3330.539		
Left S´	<10	33	8.3121	2.1984	706	0.332
	10-18	49	8.0569	2.10119		

Left E´	<10	33	14.3545	3.84058	789	0.854
	10-18	49	14.0006	4.44285		
Right S´	<10	33	5.331	3. 59409	654.5	0.144
	10-18	49	6.32	4. 23397		
Right E´	<10	33	14.7121	3.83371	674.5	0.205
	10-18	49	13.468	4.3006		
Right A´	<10	33	6.3788	2.48492	808.5	1.000
	10-18	49	6.7827	3.62784		
FS	<10	33	44.6667	4.34933	305	<0.001
	10-18	49	37.7551	5.77902		
Peak A (left)	<10	33	53.6606	10.14319	721	0.408
	10-18	49	56.9204	13.31608		
Peak E (right)	<10	33	67.8303	12.33692	784	0.817
	10-18	49	69.4837	13.08559		
	10-18	49	49.7571	9.97762		
Left E/E´	<10	33	8.1115	4.68837	730	0.458
	10-18	49	22.5339	102.23063		
Right E/E´	<10	33	5.45	3.73918	604	0.053
	10-18	49	14.5908	62.97314		
Left A/A´	<10	33	11.4691	8.83122	773.5	0.741
	10-18	49	31.3973	138.83222		
Right A/A´	<10	33	11.2797	12.63165	775.5	0.755
	10-18	49	24.2008	104.57245		
Variables	Age	Number	Mean	SD	t-test	P-value
Height	<10	33	116.36	9.598	-11.73	<0.001
	10-18	49	144.71	11.425		
Weight	<10	33	20.09	4.018	-10.72	<0.001
	10-18	49	36.63	8.213		
Left A´	<10	33	5.9576	2.08207	-0.04	0.967
	10-18	49	5.9765	1.95914		
EF	<10	33	77.0909	5.69739	5.13	<0.001
	10-18	49	69.3469	7.30169		
Peak E (left)	<10	33	101.9939	21.95632	0.09	0.926
	10-18	49	101.6	16.22476		
Peak A (right)	<10	33	50.4667	10.14417	0.31	0.755
	10-18	49	49.7571	9.97762		
Right MPI	<10	33	0.6361	0.1881	-4.23	<0.001
	10-18	49	0.8182	0.19354		
Left MPI	<10	33	0.5321	0.16701	-5.67	<0.001
	10-18	49	0.8194	0.25658		

NT-proBNP=N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A'= late diastolic velocity; S' =systolic peak velocity, EF= ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, E/E'= early diastolic pulsed-wave Doppler /early diastolic velocity, A/A'= late diastolic wave/ late diastolic velocity, MPI= myocardial performance index, Peak E= early mitral and tricuspid valve flow velocity , PeakA= late mitral and tricuspid valve flow velocity, SD= standard deviation.

Table-4: Age comparison of clinical and laboratory findings of β-thalassemia major patients for Nt-pro BNP <847.5 and Nt- pro BNP >=847.5

Variables	Age, year	Nt- pro BNP <847.5				Nt- pro BNP >=847.5			
		Mean	SD	Mann-Whitney U	P-value	Mean	SD	Mann-Whitney U	P-value
Iron	<10	229.95	73.58	208.50	0.026	275.23	50.16	99.50	0.843
	10-18	276.27	51.28			279.50	42.62		
Ferritin	<10	4352.00	5406.21	270.50	0.274	5781.31	4894.69	96.50	0.742
	10-18	4640.55	3354.22			4673.13	3390.21		
Left S´	<10	8.09	2.06	299.00	0.569	8.65	2.44	87.50	0.469
	10-18	7.95	2.48			8.28	0.98		
Left E´	<10	14.44	4.34	304.00	0.633	14.22	3.09	84.00	0.380
	10-18	13.63	4.65			14.76	4.01		
Right S´	<10	5.720	3.810	315.50	0.790	4. 732	3. 290	52.50	0.023
	10-18	5.809	4.532			7. 373	3. 434		
Right E´	<10	14.91	4.26	270.50	0.275	14.42	3.21	86.00	0.430
	10-18	13.57	4.34			13.26	4.35		
Right A´	<10	6.15	2.09	320.00	0.854	6.73	3.05	101.50	0.913
	10-18	6.74	3.67			6.87	3.66		
FS	<10	44.40	4.21	126.00	0.000	45.08	4.70	39.00	0.004
	10-18	37.00	6.12			39.31	4.80		
Peak A (left)	<10	57.19	10.15	302.50	0.614	48.23	7.66	68.50	0.119
	10-18	57.48	13.40			55.76	13.50		
Peak E (right)	<10	67.76	12.23	299.50	0.576	67.95	13.00	93.00	0.629
	10-18	71.62	13.76			65.09	10.65		
Left E/E´	<10	8.45	5.63	278.50	0.345	7.60	2.82	102.00	0.930
	10-18	29.51	124.55			8.15	4.90		
Right E/E´	<10	5.53	4.16	229.00	0.064	5.33	3.13	92.00	0.599
	10-18	18.94	76.72			5.63	2.61		
Left A/A´	<10	12.36	8.97	322.00	0.883	10.10	8.79	103.50	0.983
	10-18	40.32	168.83			13.00	18.30		
Right A/A´	<10	11.71	11.77	308.00	0.686	10.61	14.33	84.00	0.380
	10-18	31.27	127.34			9.61	7.79		
Variables	Age	Mean	SD	t-test	P-value	Mean	SD	t-test	P-value
Height	<10	116.35	9.86	-9.76	0.000	116.38	9.57	-6.65	0.000
	10-18	146.94	11.72			140.13	9.56		
Weight	<10	20.30	3.99	-8.06	0.000	19.77	4.21	-7.53	0.000
	10-18	37.52	9.01			34.81	6.11		
Age	<10	7.90	1.80	-6.62	0.000	8.00	1.87	-5.40	0.000
	10-18	12.33	2.64			13.38	3.16		
Left A´	<10	5.85	2.11	0.12	0.906	6.13	2.11	-0.34	0.738
	10-18	5.78	1.95			6.39	1.97		
EF	<10	76.20	4.77	3.87	0.000	78.46	6.86	3.20	0.004
	10-18	68.88	7.59			70.31	6.80		
Peak E (left)	<10	102.43	22.10	0.54	0.589	101.33	22.62	-0.54	0.596
	10-18	99.70	14.40			105.51	19.38		

Peak A (right)	<10	53.15	9.55	1.08	0.284	46.34	9.98	-0.72	0.476
	10-18	50.11	10.11			49.03	9.98		
Right MPI	<10	0.65	0.20	-2.87	0.006	0.61	0.17	-3.28	0.003
	10-18	0.82	0.21			0.81	0.16		
Left MPI	<10	0.55	0.18	-4.00	0.000	0.51	0.15	-4.15	0.000
	10-18	0.82	0.27			0.82	0.23		

NT-proBNP=N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A'= late diastolic velocity; S' =systolic peak velocity, EF= ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, E/E'= early diastolic pulsed-wave Doppler /early diastolic velocity, A/A'= late diastolic wave/ late diastolic velocity, MPI= myocardial performance index, Peak E= early mitral and tricuspid valve flow velocity , PeakA= late mitral and tricuspid valve flow velocity, SD= standard deviation.

Table-5: Age comparison of clinical and laboratory findings of β-thalassemia patients for E/E' < 9 and E/E' > = 9

Variables	Age, year	E/E'< 9				E/E'> = 9			
		Mean	SD	Mann-Whitney U	P-value	Mean	SD	Mann-Whitney U	P-value
Nt- pro BNP	<10	1068.00	1104.14	471.00	0.469	1357.84	1453.96	29.00	0.913
	10-18	1048.44	881.93			1017.51	1009.13		
Iron	<10	254.85	64.87	463.50	0.410	216.00	80.51	12.50	0.057
	10-18	273.08	51.74			293.90	26.54		
Ferritin	<10	5029.44	4885.18	504.00	0.769	4400.33	6877.14	21.50	0.355
	10-18	4575.38	3352.43			4946.80	3403.72		
Left S´	<10	8.84	1.07	476.00	0.510	5.95	4.10	28.00	0.828
	10-18	8.62	1.34			5.87	3.05		
Left E´	<10	15.64	2.33	512.00	0.850	8.57	4.12	25.50	0.625
	10-18	15.63	2.78			7.64	4.04		
Right S´	<10	4.942	2.860	451.50	0.326	7.080	5.972	21.00	0.328
	10-18	5.735	4.142			8.602	3.989		
Right E´	<10	15.78	2.20	451.50	0.328	9.90	5.91	25.50	0.625
	10-18	14.82	2.92			8.18	4.87		
Right A´	<10	6.63	1.58	473.50	0.489	5.27	4.97	24.50	0.550
	10-18	7.49	3.47			4.01	2.91		
FS	<10	45.41	4.10	152.50	0.000	41.33	4.18	21.50	0.355
	10-18	37.59	5.79			38.40	6.00		
Peak A (left)	<10	53.35	10.29	476.00	0.510	55.07	10.25	23.00	0.447
	10-18	56.82	13.95			57.31	11.15		
Peak E (Right)	<10	65.47	12.32	404.00	0.110	78.43	4.53	8.00	0.017
	10-18	70.81	12.41			64.31	15.04		
Right E/E´	<10	4.23	1.04	351.50	0.022	10.93	6.32	28.00	0.828
	10-18	4.97	1.33			52.12	138.48		
Left A/A´	<10	8.87	2.69	520.00	0.932	23.17	16.15	26.00	0.664
	10-18	8.91	2.53			119.11	303.36		
Right A/A´	<10	7.74	2.29	508.50	0.814	27.19	24.95	29.00	0.914
	10-18	7.60	2.77			88.95	228.99		

Variables	Age, year	Mean	SD	t-test	P-value	Mean	SD	t-test	P-value
Height	<10	114.33	8.98	-12.27	0.000	125.50	6.92	-3.69	0.002
	10-18	145.13	11.37			143.10	12.13		
Weight	<10	18.89	3.21	-13.50	0.000	25.50	2.59	-2.70	0.021
	10-18	36.90	7.38			35.60	11.33		
Age	<10	7.59	1.74	-9.43	0.000	9.50	1.22	-1.95	0.072
	10-18	13.00	2.91			11.40	2.17		
Left A´	<10	6.32	1.46	-0.73	0.471	4.32	3.57	0.47	0.643
	10-18	6.58	1.36			3.64	2.24		
EF	<10	78.04	5.48	5.36	0.000	72.83	5.00	1.28	0.220
	10-18	69.64	7.22			68.20	7.89		
Peak E (left)	<10	98.70	20.40	-0.58	0.562	116.83	24.48	1.48	0.161
	10-18	101.46	16.60			102.14	15.51		
Peak A (Right)	<10	48.86	9.60	-0.35	0.726	57.68	10.16	1.12	0.280
	10-18	49.68	8.71			50.07	14.51		
Right MPI	<10	0.62	0.15	-4.95	0.000	0.72	0.33	-0.53	0.605
	10-18	0.83	0.20			0.79	0.19		
Left MPI	<10	0.53	0.15	-5.92	0.000	0.55	0.24	-1.82	0.091
	10-18	0.83	0.26			0.77	0.24		

NT-proBNP=N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A'= late diastolic velocity; S' =systolic peak velocity, EF= ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, E/E'= early diastolic pulsed-wave Doppler /early diastolic velocity, A/A'= late diastolic wave/ late diastolic velocity, MPI=myocardial performance index, Peak E= early mitral and tricuspid valve flow velocity , PeakA=late mitral and tricuspid valve flow velocity, SD= standard deviation.

Table-6: Age comparison of clinical and laboratory findings of β-thalassemia major patients for left S’ < 8 and S’ > = 8

Variables	Age, year	Systolic peak velocity (S´) < 8				Systolic peak velocity (S´) > = 8			
		Mean	SD	Mann-Whitney U	P-value	Mean	SD	Mann-Whitney U	P-value
NT-pro BNP(pg/ml)	<10	1087.63	1142.93	85.00	0.667	1133.10	1183.51	322.00	0.797
	10-18	1113.57	950.14			988.54	871.06		
Iron	<10	208.22	74.19	46.00	0.028	262.63	61.08	318.50	0.748
	10-18	286.24	37.04			270.64	54.82		
Ferritin	<10	6411.33	7348.51	93.50	0.964	4353.96	4166.10	275.00	0.263
	10-18	4397.52	3225.61			4841.43	3453.31		
Left E´	<10	12.22	5.93	85.00	0.667	15.15	2.42	271.50	0.236
	10-18	11.70	4.99			15.73	3.07		
Right S´	<10	7.331	4.795	84.00	0.634	4. 580	2. 797	283.00	0.329
	10-18	7.592	4. 706			5. 365	3. 641		
Right E´	<10	12.47	5.93	77.50	0.442	15.55	2.34	329.50	0.905
	10-18	11.24	4.90			15.14	2.89		
Right A´	<10	4.77	2.86	91.00	0.874	6.98	2.09	256.50	0.144
	10-18	4.94	2.36			8.17	3.83		

FS	<10	43.67	4.06	44.00	0.022	45.04	4.48	108.50	0.000
	10-18	38.48	5.58			37.21	5.97		
Peak A (left)	<10	53.30	11.33	93.50	0.964	53.80	9.92	262.50	0.177
	10-18	53.44	12.42			59.53	13.58		
Peak E (right)	<10	71.79	11.41	77.00	0.428	66.35	12.57	283.00	0.330
	10-18	69.29	14.60			69.63	12.10		
Left E/E´	<10	11.78	7.74	89.00	0.803	6.74	1.59	325.00	0.840
	10-18	43.68	155.75			6.68	1.73		
Right E/E´	<10	8.34	6.28	76.00	0.402	4.37	1.11	284.00	0.340
	10-18	27.60	95.93			4.84	1.59		
Left A/A´	<10	18.92	14.51	86.00	0.700	8.68	2.32	330.00	0.912
	10-18	61.41	211.18			8.89	2.27		
Right A/A´	<10	21.08	21.83	92.00	0.910	7.60	2.14	292.50	0.425
	10-18	47.04	158.99			7.07	2.50		
Variables	Age(year)	Mean	SD	t-test	P-value	Mean	SD	t-test	P-value
Height	<10	121.78	8.45	-4.05	0.000	114.33	9.36	-12.89	0.000
	10-18	140.14	12.37			148.14	9.50		
Weight	<10	21.56	3.94	-3.96	0.000	19.54	3.99	-11.64	0.000
	10-18	34.14	9.11			38.50	7.07		
Age	<10	8.78	1.20	-3.55	0.001	7.63	1.91	-7.80	0.000
	10-18	12.43	2.96			12.86	2.77		
Left A´	<10	4.54	2.68	-0.27	0.786	6.49	1.57	-0.95	0.346
	10-18	4.79	2.10			6.86	1.29		
EF	<10	75.89	5.82	2.64	0.013	77.54	5.71	4.12	0.000
	10-18	68.71	7.17			69.82	7.49		
Peak E (left)	<10	106.40	27.07	0.51	0.614	100.34	20.13	-0.17	0.864
	10-18	102.15	17.86			101.19	15.20		
Peak A (right)	<10	51.51	12.08	0.53	0.602	50.08	9.58	-0.05	0.957
	10-18	49.14	10.95			50.22	9.36		
Right MPI	<10	0.70	0.27	-0.93	0.359	0.61	0.15	-4.64	<0.001
	10-18	0.78	0.16			0.85	0.21		
Left MPI	<10	0.59	0.22	-1.71	0.099	0.51	0.14	-6.96	<0.001
	10-18	0.80	0.33			0.84	0.19		

NT-proBNP=N-terminal prohormone of brain natriuretic peptide, E' = early diastolic velocity; A'= late diastolic velocity; S' =systolic peak velocity, EF= ejection fraction, FS = fractional shortening, E = early diastolic pulsed-wave Doppler, A = late diastolic wave, E/E'= early diastolic pulsed-wave Doppler /early diastolic velocity, A/A'= late diastolic wave/ late diastolic velocity, MPI=myocardial performance index, Peak E=early mitral and tricuspid valve flow velocity , PeakA=late mitral and tricuspid valve flow velocity, SD= standard deviation.

4- DISCUSSION

One of the most important organs that impressed by iron overload is heart especially in thalassemia patients. The main causes of thalassemia cardiomyopathy are increasing intestinal absorption hemolysis and lifelong blood transfusions determine myocardial iron overload (16). Ibrahim et al. (17) conducted a study on pediatric thalassemia compared with control and concluded that ferritin was significantly varied between groups, but EF and FS were same. For the right and left DTE, they observed a significant difference. Barbero et al. (18) applied DTE to evaluate MPI in thalassemia and resulted approximately all right heart functions were same in patients and controls; but some of predominant left hear functions such as MPI and S' were dissimilar significantly. The current study mostly found same results especially in DTE findings with these studies. Gupta et al. applied DTE and found comparable results with the current results in the case of left heart functions by DTE (S', E', A' and E'/A') that revealed similarity except E' (19). Garadah et al. (4) assessed systolic and diastolic functions of the left ventricle and found that diastolic indices of LV in patients showed higher early diastolic filling of LV, and E/A ratio suggesting restrictive diastolic pattern and stiff myocardial wall in patients. Similarly, Yaprak et al. (20) demonstrated that patients had significantly higher E wave, E/A ratio, and lower A wave velocity that suggesting a restrictive pattern but with no correlation with hemoglobin level.

Spirit et al. (21) demonstrated a restrictive pattern in patients with no heart failure. This was in agreement with a previous report that high E/A ratio was the most common finding. Kremastinos et al. (3) observed lower systolic and diastolic velocity waves and E/E' in the patients' group while FS and EF were same in groups. They also demonstrated that NT-pro BNP was increased significantly in the third decade of life in patients, whereas E/E′ ratio increased in the fourth decade. Maximal left atrial and right ventricular diameters were also increased in the patients group and mitral E-wave velocity while no statistical difference was seen in A wave and E/A ratio. In the study E wave deceleration time was decreased in patients and finally they introduced NT-pro BNP as an earlier biomarker of LV diastolic dysfunction (2). Many studies implicated to this fact that NT-pro BNP is a sensitive biomarker to detect asymptomatic LV dysfunction especially with an important role in diagnostic and prognostic thalassemia implications (2, 3, 14). In these studies, NT-pro BNP levels were correlated with the presence of diastolic LV dysfunction in thalassemia patients. Kremastinos et al. (2) proposed an increase of NT-pro BNP in thalassemia patients and reported that this increase correlated with age and LV diastolic dysfunction but Balkan et al. (5) did not find a significant correlation. Current study revealed that some of heart dysfunctions values changed with the level of NT-pro BNP variation, this variation was more affected by age changes in every level of NT-pro BNP.

These heart dysfunctions would be introduced as: Left and right MPI as diastolic and systolic dysfunctions, right S' as systolic dysfunction as well as FS and EF. As observed, in the present study the number of heart dysfunctions that changed with the NT-pro BNP variation was very low that could be similar with Balkan study. The probable reason for the lack of correlation in Balkan study may be was due to mean age of their patients that was lower and the range of age was narrow. The results of the current study demonstrated that NT-pro BNP, Iron, Ferritin were higher in thalassemia patients and variables of left S', left and right A', EF, FS were lower in patients and left and right A/A', left and right MPI were higher.

In regards to the lower level of NT- pro BNP than cutoff point, older patients had higher levels of MPI in both left and right and Iron level as well when EF and FS were lower. Left systolic peak velocity (S') was more valuable compared to the right S' to detect systolic dysfunction. FS and EF were lower in old patients in both levels of NT- pro BNP and right and left MPI levels were higher. The most important finding of these above studies was an increase of NT-pro BNP levels in patients with no signs or symptoms of HF compared to the healthy controls similar to the previous studies (2, 3). Aessopos et al. have found high levels of BNP only in patients with overt heart failure, independently of severity of cardiac insufficiency (6, 16). The patient was divided into two groups according to E/E' ratio: E/E' < 9 and E/E' ≥ 9. Patients with an E/E' ratio < 9 were considered as having normal diastolic dysfunction, whereas patients with an E/E' ratio ≥ 9 were considered as patients with suspected diastolic dysfunction. Kremastinos et al. (2) divided the study patients in three groups based on 8 and 15 cutoff points of left E/E'. In these three groups of patients, they detected that hemoglobin levels were similar between groups, while age and total transfused blood units showed an increase in the groups with a higher E/E′ ratio. Between patients' groups they found A' and DT were same and E, E/A, NT pro BNP and ferritin had different values so that NT-pro BNP was higher significantly in patients with higher level of E/E'.

For more evidences on effect of aging on NT-pro BNP levels and diastolic dysfunction Kremastino divided the patient population into the first five decades. Both NT-pro BNP levels and E/E′ ratio showed a statistically significant increase throughout patients' life. NT-pro BNP levels appear to increase earlier, in the third decade of life, being significantly higher than levels in the 1st or 2nd decades. On the other hand, E/E′ ratio seems to increase later in life, being significantly higher only in the 4th decade. They expressed that although NT-pro BNP levels were increased in patients with higher E/E′ ratio this increase only became statistically significant in the 3rd decade of life, while E/E′ ratio increased in the 4th decade. We also detected that NT- pro BNP increased by age similar with the Kremastinos et al. study (2). The current study detected that the left and right S', A' and A/A' had significant different values in all age groups except those were lower than 10 years. The levels of Left and right E' were different significantly in all age groups. Left and right A' levels were different. Patients had different levels of right E/E' significantly in all age groups except the age group of higher than 20 years. In another study, Kremastinos also found that an increase of NT-pro BNP levels in patients with beta thalassemia major was related to age and diastolic dysfunction (E/E' ratio >15) and the highest decade in the study (3). Therefore the results of the studies by Kremastinos et al. showed the similar pattern that E/E' ratio and NT- pro BNP were increased by age. From the Balkan et al. (5) study resulted that NT-pro BNP levels were higher in patients with E/E < 9 compared to controls. They also concluded that NT-pro BNP levels were higher in patients with E/E' ≥ 9 compared to those with E/E'< 9, but this increase was not statistically significant. Same trends resulted for E, left ventricular end systolic diameter, left ventricular end diastolic diameter, LA diameter by Echocardiographic evaluation.

In the present study, despite of difference between types of echocardiography variables, the results in general were similar with Balkan's study. These results deal to this fact that an increase in the ratio of left E/E' causes diastolic dysfunction in the patients aged < 20 years especially when the level of E/E' is more than suspected level. Balkan et al. (5) reported that NT-pro BNP levels were significantly higher in patients with E/E' ratio < 9 in comparison to healthy controls. Echocardiographic evaluation revealed that E, LVES, LVED, LA diameter, LAVI, and LVMI values were significantly higher in E/E' ratio ≥ 9 than E/E' ratio < 9. Our results revealed that according to the cutoff point 9 for left E/E', EF and FS were lower in older patients when E/E' had lower level. Right and left MPI had positive association with age when E/E' < 9. Few studies only included E and A velocities and E/A ratio as a surrogate markers of diastolic functions (5) whereas some studies included that tissue Doppler imaging (TDI), and strain imaging methods for the estimation of diastolic filling pressures (22). On the hypothesis that longitudinal ventricular functions may give early information about impending cardiac dysfunction, the current study used TDI to evaluate systolic (left S') and diastolic velocity (left E', left A' and left E/E' ratio), and observed that Iron had higher concentration in old patients when left S' < 8. In both levels of left S' (S' > 8 cm/s and S' < 8 cm/s), EF had lower level in older patients. Right and left MPI were higher in patients aged more than 10 years in both categorization of left S'.

Marcello Marc et al. (23) performed a study on patients with thalassemia to detect early cardiac dysfunction by DTE and resulted that patients with reduced systolic peak velocity (left S' < 8 cm/s) had a mild abnormality of early diastolic velocity (left E') in comparison to patients with normal systolic velocity (left S' > 8 cm/s). With similar classification of left S', the current study resulted that left E' was similar in both groups of patients even for the considered age groups. Same pattern observed for L.A. diameter, left ventricular dimensions, E and A peak velocities/A ratio, Am, and E/E' ratio in the two groups. In compare with our results, would be observed that the results are similar and in same line with our results related to EF Marcello Marc detected lower level for EF in the second group, although values were within the normal range in both groups. Same results observed from the study conducted by Kremastinos et al. (24) who found diastolic abnormalities only after onset of cardiac failure. The results of these two studies are consisted with the current study results especially for those with systolic peak velocity less than 8 cm/s. In the present study peak systolic velocity (S') was significantly reduced in patients. Also, mentioned that a peak systolic velocity < 8 cm/s allows identifying a group of patients at higher risk of cardiac adverse events. It is of interest that EF was not significantly reduced in all age groups of patients with Sm < 8 cm/s, dissimilar with Marcello Marc findings. These findings also are dissimilar with Akpinar et al. (25). Many studies demonstrated impairment of peak systolic velocity (S'), despite a normal EF. Furthermore our results corroborate findings of other recent researches which have demonstrated a pivotal role of TDI in detecting early myocardial damage in such patients. Recently Marcí et al. and Hamdy et al. have reported early impairment of longitudinal systolic function, detected by TDI, in thalassemia patients without heart failure (10, 11, 23).

4-1. Limitations of the study

The study limitation was lack of Proper Corporation by participants especially controls.

5- CONCLUSION

The study confirmed that NT-pro BNP increases in thalassemia patients and is related to age and LV diastolic dysfunction. NT-pro BNP with E/E' and S' were shown diastolic and systolic dysfunction in patients with major thalassemia. We presented the relation between plasma NT-pro BNP levels, iron overload, and various echocardiography indices of myocardial dysfunction in young thalassemia patients. While there was a strong correlation between plasma NT-proBNP levels and iron overload. There was no correlation between NT-proBNP levels and diastolic dysfunction parameters in patients in the second decade of life. An increased level of ferritin and NT-pro BNP can be used as a marker for the intensification of iron chelation therapy, which reverses iron-induced cardiomyopathy.

6- AUTHORSHIP CONTRIBUTION

Noor Mohammad Noori designed the study. Data analyzed by Alireza Teimouri as well as writing the primary manuscript and corresponding author. The critical revision supervised by Noor Mohammad Noori and Alireza Teimouri.

7- CONFLICT OF INTEREST: None.

8- ACKNOWLEDGMENT

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

9- REFERENCES

1. Noori NM, Mahjoubifard M, Mohammadi M, Fard AJ, 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. Iranian Red Crescent Medical Journal 2014; 16(5):e11698.

2. Kremastinos DT, Tsiapras DP, Kostopoulou AG, Hamodraka ES, Chaidaroglou AS, Kapsali ED. NT-proBNP levels and diastolic dysfunction in β-Thalassaemia major patients. European journal of heart failure. 2007; 9(5):531-6.

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

4. Garadah T, Kassab S, Mahdi N, Abu-Taleb A, Jamsheer A. Pulsed and Tissue Doppler Echocardiographic Changes in Patients with Thalassemia Major. Clinical Medicine: Blood Disorders. 2010; 3:1–8.

5. Balkan C, Tuluce SY, Basol G, Tuluce K, Ay Y, Karapinar DY, Gurgun C, Bayindir O, Kavakli K. Relation between NT-proBNP Levels, Iron Overload, and Early Stage of Myocardial Dysfunction in β-Thalassemia Major Patients. Echocardiography. 2012; 29(3):318-25.

6. Aessopos A, Farmakis D, Polonifi A, et al: Plasma B-type natriuretic peptide concentration in beta-thalassaemiapatients. Eur J Heart Fail 2007; 9: 537–54.

7. Tang WH, Girod JP, Lee MJ, et al: Plasma B-type natriuretic peptide levels in ambulatory patients with establishedchronic symptomatic systolic heart failure. Circulation 2003; 108: 2964–66.

8. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004; 350: 655-63.

9. Akpinar O, Acarturk E, Kanadasi M, et al: Tissue Doppler imaging and NT-proBNP levels show the early impairment of ventricular function in patients with betathalassemia major. Acta Cardiol 2007; 62:225–31.

10. Hamdy AM. Use of strain and tissue velocity imaging for early detection ofregional myocardial dysfunction in patients with beta thalassemia. Eur J Echocardiography 2007; 8:102–9.

11. Hamdy AM, Zein El-Abdin MY, Abdel-Hafey MA. Tissue Doppler has an important prognostic value in patients with beta thalassemia. Echocardiography 2008; 25:554–55.

12. Noori N, Mohamadi M, Keshavarz K, Alavi S, 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. 2015; 8(1):35-41.

13. Noori NM, Keshavarz K, Shahriar M. Cardiac and pulmonary dysfunction in asymptomatic beta-thalassanemia major. Asian Cardiovascular and Thoracic Annals. 2012; 20(5): 555-9.

14. 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.

15. M, Totaro A, Ieva R, Brunetti ND, Di Biase M. Time intervals and myocardial

performance index by tissue Doppler imaging. Internal and Emergency Medicine. 2011; 6(5):393-402.

16. Aessopos A, Berdoukas V, Tsironi M: The heart in transfusion dependent homozygous thalassaemia today prediction, prevention and management. Eur J Haematol 2008; 80:93–106.

17. Ibrahim MH, Azab AA, Kamal NM, Salama MA, Ebrahim SA, Shahin AM, et al. Early detection of myocardial dysfunction in poorly treated pediatric thalassemia children and adolescents: Two Saudi centers experience. Annals of Medicine and Surgery. 2016; 9: 6-11.

18. Barbero U, Longo F, Destefanis P, Gaglioti CM, Pozzi R, Piga A. Worsening of myocardial performance index in beta-thalassemia patients despite permanently normal iron load at MRI: A simple and cheap index reflecting cardiovascular involvement? IJC Metabolic and Endocrine. 2016; 13:41-4.

19. Gupta A, Kapoor A, Phadke S, Sinha A, Kashyap S, Khanna R, Kumar S, Garg N, Tewari S, Goel P. Use of strain, strain rate, tissue velocity imaging, and endothelial function for early detection of cardiovascular involvement in patients with beta-thalassemia. Annals of Pediatric Cardiology. 2017; 10(2):158.

20. Yaprak I, Aksit S, Ozturk C, Bakiler AR, Dorak C, Turker M. Left ventricle diastolic abnormalities in children with beta-thalassemia major: Doppler echocardiographic study. Turk J Pediatr. 1988; 40(2):201–9.

21. Spirit P, Lupi G, Melevendi C, Veccio C. Restrictive diastolic abnormalities identified by Doppler echocardiography in patients with thalassemia major. Circulation. 1990; 82(1)88–94.

22. Bilge AK, Altinkaya E, Ozben B, et al: Early detection of left ventricular dysfunction with strain imaging in thalassemia patients. Clin Cardiol 2010; 33: 29–34.

23. Marcí M, Pitrolo L, Lo Pinto C, Sanfilippo N, Malizia R. Detection of early cardiac dysfunction in patients with Beta thalassemia by tissue Doppler echocardiography. Echocardiography. 2011; 28(2):175-80.

24. Kremastinos D, Tsiapra DP, Tsetsos GA, et al: Left ventricular diastolic Doppler characteristics in beta-thalassemiamajor. Circulation 1993; 88: 1127–35.

25. Akpinar O, Acart¨urk E, Kanadasi M, et al: Tissue Doppler imaging and NT-proBNP levels show the early impairmentof ventricular function in patients with β- thalassaemia major. Acta Cardiol 2007; 62: 225–31.