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ORIGINAL RESEARCH

The pro-BNP Serum Level and Echocardiographic Tissue Doppler Abnormalities in Patients with Beta Thalassemia Major

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Abstract:
Background: Doppler echocardiographic studies of the left ventricle (LV) function in patients with β-Thalassemia Major (β-TM) had shown different patterns of systolic and diastolic dysfunctions associated with abnormal serum brain natriuretic peptide (BNP).
Aim: This cross-sectional study was designed to study the LV systolic and diastolic functions and correlate that with serum level of N-terminal pro brain natriuretic hormone (NT- pro BNP) in patients with β-TM using Pulsed Doppler (PD) and Tissue Doppler (TD) echocardiography.
Methods: The study was conducted on patients with β-TM (n = 38, age 15.7 ± 8.9 years) and compared with an age-matched controls (n = 38, age 15.9 ± 8.9 years). In all participants, PD and TD echocardiography were performed and blood samples were withdrawn for measuring the serum level of NT-pro BNP, ferritin, and alanine transaminase.
Results: Patients with β-TM compared with controls, have thicker LV septal wall index (0.65 ± 0.26 vs. 0.44 ± 0.21 cm, $P < 0.001$), posterior wall index (0.65 ± 0.23 vs. 0.43 ± 0.21 cm, $P < 0.01$), and larger LVEDD index (4.35 ± 0.69 vs.3.88 ± 0.153 mm, $P < 0.001$). In addition, β-TM patients have higher transmitral E wave velocity (E) (70.818 ± 10.139 vs. 57.532 ± 10.139, p = 0.027) and E/A ratio (1.54 ± 0.17 vs. 1.23 ± 0.19, $P < 0.01$) and shorter deceleration time (DT) (160.13 ± 13.3 vs. 170.50 ± 19.20 m sec, $P < 0.01$). Furthermore, the ratio of transmitral E wave velocity to the tissue Doppler E wave at the basal septal mitral annulus (E/Em^-) was significantly higher in β-TM group (19.6 ± 2.81 vs. 13.868 ± 1.41, $P < 0.05$). The tissue doppler systolic wave (Sm) velocity and the early diastolic wave (Em) were significantly lower in β-TM group compared to controls (Sm: 4.82 ± 1.2 vs. 6.22 ± 2.1 mm/sec, $P < 0.05$; Em: 3.51 ± 2.7 vs. 4.12 ± 2.5 mm/sec $P < 0.05$, respectively). The tricuspid valve velocity was significantly higher in β-TM patients compared with controls (2.993 ± 0.569 vs. 1.93 ± 0.471 m/sec, respectively, $P < 0.01$). The mean serum NT pro-BNP in β-TM was significantly higher compared with controls (37.6 ± 14.73 vs. 5.5 ± 5.4pg/ml, $P < 0.05$). The left ventricle ejection fraction (EF%) and fractional shortening (FS%) were not significantly different between both groups.
Conclusion: We conclude that patients with β-TM had a significantly higher serum level of NT-pro BNP that is positively correlated with the E/Em ratio on tissue Doppler. Furthermore, we confirm our previous findings that patients with β-TM exhibit LV diastolic pattern on echocardiogram suggestive of restrictive type with well preserved left ventricle systolic function.

Keywords: NT- pro BNP, beta-thalassemia major, pulsed echo Doppler, tissue Doppler echocardiography, Bahrain

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Introduction

Patients with beta-thalassemia major (β-TM) are maintained on continuous blood transfusion regimens to keep hemoglobin levels close to normal and allow adequate tissue oxygenation. Hemolysis of red blood cell and repeated blood transfusion in β-TM is associated with iron overload.[1] Iron deposition adversely affect both the structure and function of the heart and other vital organs.[2] Treatment with iron chelating therapy in patients with β-TM showed improvement of morbidity and increased survival and is considered the standard of care of this blood disorder.[3]

Brain natriuretic peptide (BNP) is a 32-amino acid polypeptide secreted primarily by the left ventricle (LV) in response to increased stretching or wall tension of the heart muscle cells. At the time of BNP release, the inactive NT-pro BNP is cleaved from the precursor peptide pro BNP and is co-secreted in quantities directly proportional to its biologically active counterpart (BNP).[4] Both BNP and NT-pro-BNP have been shown earlier to be sensitive biomarkers for the detection of asymptomatic LV dysfunction and that they had important diagnostic and prognostic implications in patients with LV dysfunction.[5] The prognostic predictive value of NT-pro BNP in patients with thalassemia major as a model disease with isolated diastolic dysfunction has been assessed where there were a direct relationship between the diastolic indices on echo Doppler and the serum level of the NT-pro BNP.[6,7]

LV diastolic filling patterns have been classified into normal, restrictive and abnormal relaxation patterns on the basis of early filling E wave and late filling A wave on the pattern of LV diastolic filling.[8] The filling pattern depends on the degree of predominance of the abnormal active relaxation or altered wall stiffness. The restrictive pattern is characterized by high E wave velocity, decreased A wave, shortened deceleration Time (DT) with high E/A ratio.[9–12]

The tissue Doppler echocardiography (TD) can detect regional myocardial diastolic dysfunction even in early phases of cardiac injury.[13] The tissue velocity at the basal septum of mitral annulus has been evaluated as a marker of myocardial stiffness, and in one report it was suggested that the ratio of early diastolic filling wave (E) to mitral annulus velocity (Em) does correlates positively with the LV end-diastolic pressure (LVEDP).[14] Furthermore, it was shown that TD velocities of the early diastolic wave (Em) and systolic wave (Sm) were both reduced in stiff hypertensive myocardium.[15]

The aim of this study is to examine the relationship between the serum level of NT-pro BNP and LV diastolic function using different echocardiographic indices of tissue Doppler echocardiogram in patients with β-TM in the absence of overt heart failure.

Material and Methods

This study included 38 patients with transfusion-dependent β-TM and 32 healthy individuals who were used as a control group. A constitutional ethical approval was obtained for the study.

The study was conducted over six month period from September 2009 to March 2010.

Patients' selection was consecutive from those who are on regular follow-up in the Pediatric Hematology clinic at Salmaniya Medical Complex (SMC) in Bahrain.

Inclusion and exclusion criteria

Patients were included if they had a follow-up for more than 3 years at SMC, with a confirmed diagnosis of β-TM based on electrophoresis. Each patient had been receiving blood transfusions every three weeks to maintain hemoglobin levels above 9 g/dl since infancy. All patients had also been receiving oral deferasirox as a chelating therapy (20 mg/kg/day) regularly for at least six months. In the control group, individuals were healthy with no renal or hepatic diseases, no cardiac valve diseases, normal LV function on echo and negative blood tests for thalassemia. The control group was selected from the pool of age-matched healthy individuals who were referred for evaluation of a systolic murmur and turned out to have normal echo.

The blood sampling in the β-TM group and the echo test were carried out at the end of the week prior to the blood transfusion. Patients were excluded if they have an end-stage renal disease with creatinine clearance $<30\%$ of normal, severe liver disease, diabetes mellitus, hypoparathyroidism, advanced heart failure, hypertrophic cardiomyopathy or if the patient is taking active cardiac medications.

Clinical and biochemical variables

Each patient in the study had a clinical and hematological data file including duration of disease, cardiovascular assessment for pulse rate, jugular venous pressure wave, apex beat, blood pressure, heart sounds and any murmurs, ankle edema and body weight. Data of the blood level of hemoglobin, serum level of NT-pro BNP, serum ferritin, alanine transferase and creatinine were all recorded.

Doppler echocardiography

Each patient in the study had the echo examination by 2.5–5 MHz transducer, using HP E33 echo machine. The echocardiography tests were performed by an echo technologist who was blinded about the clinical condition of the patient. Data were reported as an average of at least five cardiac cycles. Another echo technician analyzed the data blindly, and the data were taken as an average of two readings. All measurements were conducted according to the recommendations of the American Society of Echocardiography (ASE).[16]

Each patient enrolled in the study had echocardiographic measurements including M-mode, 2D echo, color flow, LV systolic and diastolic transmitral indices and tissue Doppler evaluation of the septal mitral annulus. The M-mode echo parameters including the LV septal wall thickness, posterior LV wall thickness, mid and basal LV cavity dimension in systole and diastole as well as the LV end-diastolic volume (EDV). LV fractional shortening (FS%) and LV ejection fraction percentage (LVEF%) were measured using Teichholz formula: $V = \{7.0/(2.4 + D) * D^3\}$.[17] Pulsed Doppler was acquired in the apical view, while patients in the partial left lateral decubitus position during the end of expiration, with sample volume situated between the mitral leaflet tips.

The LV diastolic filling indices including early diastolic wave (E wave), late diastolic wave (A wave), E wave to A wave (E/A) ratio, deceleration time of E wave (DT) and isovolumic relaxation time (IVRT) were all measured with the sample volume at the tip of the mitral leaflets in the apical view. The tricuspid valve velocity in systole (cm/s) was measured in apical view. The tissue Doppler velocity of the septal mitral annulus, early filling E wave velocity (Em), the calculated E/Em ratio and the systolic wave (Sm) were all were recorded as a mean of five readings.

Statistical analysis

All data were entered and analyzed using the Statistical Package of Social Sciences (SPSS) version 17.0. Data are presented as mean ± SD. Unpaired *student t-test* was used to analyze the differences between the variables in the control and the β-TM groups. The M-mode dimensions of the LV were adjusted and indexed for Body Surface Area (BSA) of each patient. Linear regression analysis was used to assess the correlation between the serum NT- pro BNP and E/Em ratio. The correlation coefficient (r) of the slope was then calculated. The mean of tissue Doppler indices taken at septal mitral annulus ring of the LV were corrected for heart rate.

All other pulsed and tissue Doppler indices for systolic and diastolic functions were corrected for heart rate such as: E wave velocity, A wave velocity, DT, IVRT, Em, E/Em, Sm. Differences between groups were considered statistically significant at a probability value of <0.05.

Results

The demographic data of β-TM patients and the healthy controls are summarized in Table 1. The β-TM patients had a significantly lower body surface area compared with control patients (1.02 ± 0.2 vs. 1.15 ± 0.2, $P < 0.05$). There was no significant difference in the mean age between the β-TM group (15.92 ± 8.92 year, range; 7–25) and the control group (15.79 ± 8.94 years, range; 6–24) with comparable gender distribution between the two groups.

Table 2 shows the hemodynamic and biochemical variables in both groups. The systolic and diastolic pressure values were comparable in both groups. The heart rate was lower in the β-TM compared with control group (68.16 ± 7.40 vs.72.92 ± 6.08 bpm respectively, $P < 0.05$). The mean serum NT

Table 1. Demographic characteristics of control (n = 32) and thalassemia patients (n = 38) in the study.

	Control group (n = 32)	β-TM group (n = 38)	*P* value
Age (Years)	15.79 ± 8.94	15.92 ± 8.92	0.92
Male	22 (57%)	23 (60%)	0.75
Weight (kg)	41.26 ± 8.60	31.00 ± 7.87	0.034
Height (cm)	135.66 ± 8.80	134.87 ± 9.74	0.001
BSA	1.15 ± 0.20	1.02 ± 0.20	0.001

Notes: Data are presented as mean ± SD. *P* value < 0.05 is considered statistically significant.
Abbreviation: BSA, body surface area.

Table 2. Hemodynamic and biochemical parameters in beta thalassemia (β-TM) patients on deferasirox (20 mg/kg/day) and the control group.

Parameter	Control group (n = 32)	β-TM group (n = 38)	*P* value
Systolic pressure (mmHg)	128.16 ± 12.49	123.34 ± 13.27	0.108
Diastolic pressure (mmHg)	74.71 ± 7.61	71.26 ± 7.21	0.102
Heart rate (bpm)	72.92 ± 6.08	68.16 ± 7.40	0.04
Serum pro-BNP (pg/ml)	5.53 ± 5.41	37.62 ± 14.73	0.001
Serum ferritin (μg/L)	164 ± 48	5124 ± 1931	0.001
Serum creatinine (μmol/l)	80.80 ± 10.12	75.23 ± 11.13	0.076
Serum alanine transaminase (U/L)	39 ± 10.72	40.45 ± 14.83	0.085
Hemoglobin (gm/dl)	10.23 ± 0.51	9.5 ± 0.42	0.065

Notes: Data are presented as mean ± SD. $P < 0.05$ is considered statistically significant.

pro-BNP in β-TM was significantly higher compared with controls (37.62 ± 14.73 pg/ml versus 5.53 ± 5.41pg/ml respectively, $P < 0.001$). The serum ferritin level was significantly higher in the β-TM group compared with the control group (5124 ± 1931 pg/ml vs. 164 ± 48 pg/ml, $P < 0.001$). There were no significant differences in serum levels of creatinine, alanine transaminase and hemoglobin between the two groups.

Echocardiographic findings

Table 3 summarizes the indexed pulsed M-mode values in both control and β-TM groups. The β-TM group showed significantly higher LV wall thickness manifested by significantly thicker posterior wall and inter-ventricular septum ($P < 0.001$ in both). The LV dimensions at the end of systole (LVESD) and diastole (LVEDD) were significantly larger in β-TM compared with control group. The left ventricle mass was significantly higher in β-TM group compared with the control (172.85 ± 65.30 vs. 87.65 ± 14.76 respectively, $P < 0.001$).

Compared with the control group, patients with β-TM had slightly higher LVEF% and FS, but these differences did not achieve the statistical significance.

Table 4 shows the pulsed and tissue Doppler indices of the LV in both groups. The pulsed Doppler velocities of LV in diastolic showed significantly higher E wave, and E/A ratio in β-TM group compared to control while significantly shorter interval of DT time and higher velocity of E wave. The ratio of transmitral E wave velocity to TD (Em) of the septal mitral annulus (E/Em) was significantly higher in the β-TM group compared to the control group ($P < 0.05$), while the early diastolic velocity (Em) and the systolic wave velocity (Sm) were significantly lower in β-TM group ($P < 0.05$).

Table 3. Indexed M-Mode and 2D echocardiography findings in beta thalassemia (β-TM) patients and the control group.

Parameter	Control group (n = 32)	β-TM group (n = 38)	*P* value
IVS (cm/m^2)	0.44 ± 0.22	0.65 ± 0.26	<0.001
PW (cm/m^2)	0.43 ± 0.21	0.65 ± 0.24	<0.001
LVEDD (mm/m^2)	3.89 ± 0.15	4.35 ± 0.70	<0.001
LVESD (mm/m^2)	2.72 ± 0.17	2.85 ± 0.47	<0.01
LV mass (gm/m^2)	87.65 ± 14.76	172.85 ± 65.30	<0.001
LVEF (%)	59.00 ± 3.33	63.47 ± 6.76	0.089
FS (%)	32.37 ± 4.36	34.41 ± 3.36	0.107

Notes: Data are presented as mean ± SM per body surface area (m^2). $P < 0.05$ is considered statistically significant.
Abbreviations: IVS, inter ventricular septal thickness; PW, posterior wall thickness; LVEDD, left ventricle end diastolic diameter; LVESD, left ventricle end systolic diameter; FS, fractional shortening; LVEF, left ventricular ejection fraction.

Table 4. The heart rate corrected diastolic Doppler indices for β-thalassemia (β-TM) patients.

Parameter	Control group (n = 32)	β-TM group (n = 38)	*P* value
Mitral inflow velocities			
E (cm/sec)	57.53 ± 10.14	70.82 ± 10.1	0.027
A (cm/sec)	46.34 ± 10.76	45.53 ± 9.82	0.109
E/A ratio	1.23 ± 0.17	1.54 ± 0.18	0.001
DT (msec)	170.50 ± 19.20	160.53 ± 13.30	0.047
IVRT (msec)	79.50 ± 5.72	75.21 ± 6.37	0.083
Doppler tissue imaging			
Em (cm/s)	4.12 ± 2.50	3.51 ± 2.70	0.04
Sm (cm/s)	6.22 ± 2.10	4.82 ± 1.20	0.02
E/Em ratio	13.86 ± 1.41	19.68 ± 2.81	$<$0.05
Tricuspid velocity (cm/sec)	1.74 ± 0.47	2.85 ± 0.57	0.045

Notes: Data are presented as mean ± SD. $P < 0.05$ is considered statistically significant.
Abbreviations: IVRT, isovolumic relaxation time; E, early diastolic flow velocity; A, late diastolic flow velocity; DT, deceleration time of flow velocity in early diastole; Sm, systolic myocardial velocities of basal segments of LV lateral wall; Em, early diastolic myocardial velocities of the same basal segments.

The LV fractional shortening and LVEF% at mid-cavity sector were with not significantly different between the two groups.

There was a significant positive correlation between the pro BNP and the E/Em ratio (r = 0.31, $P < 0.001$).

The tricuspid valve velocity was significantly higher in β-TM group ($P < 0.01$). In patients with β-TM group, the color flow and continuous wave velocity of the LV and RV showed mild mitral regurgitation in nine patients and three patients had mild aortic regurgitation with none in the control group.

Discussion

In this study, patients with β-TM were evaluated with pulse and tissue Doppler echocardiogram for systolic and diastolic functions of the left ventricle (LV) compared with age and sex-matched individuals with no β-TM (controls). Compared with controls, the diastolic indices of LV in β-TM patients showed higher early diastolic filling of LV and E/A ratio suggesting restrictive diastolic pattern and stiff myocardial wall. These findings are in keeping with one study by Yaprak et al.[18] who demonstrated that β-TM patients had significantly higher E wave, E/A ratio, and lower A wave velocity, suggesting a restrictive pattern but with no correlation with hemoglobin level. Similar observations were reported by Spirit et al.[19] who demonstrated a restrictive pattern in patients with β-TM with no heart failure. This was also in agreement with a previous report that high E/A ratio is the most common finding in patients with β-TM.[20]

In one study, the magnitude of E wave velocity is governed by the initial left ventricular pressure and shown to be directly related to it,[21] the decreased DT of E wave was mostly related to the increased amplitude of the E wave and may be due to the impaired relaxation of LV with a constrictive pattern. The exact mechanism of these findings is not known but may be due to iron overload and increased stiffness of LV wall.

The tissue Doppler ratio of E/Em was significantly higher in β-TM patients compared with controls. Likewise, the early diastolic velocity (Em) and the systolic velocity wave (Sm) of the basal septum were significantly reduced, suggesting stiff myocardium and possibly high LVEDP. These findings are in agreement with those previously reported in hypertensive patients.[22,23]

The LVEF% and fractional shortening in mid cavity sector were normal indicating that β-TM patients are having a preserved systolic function. In the current study, the serum ferritin was more than 30 times higher in the β-TM group compared with controls. These findings confirm our previously published studies that iron overload appear to mediate the impaired diastolic function leading to stiffness of the myocardial wall but with well preserved LV systolic function.[24] However, increased serum ferritin concentration may not be associated with a concordant and comparable increase in myocardial iron content. Future studies which examine the cardiac iron content by using more sensitive measures such as MRI will be required.

In this study, the serum level of pro-BNP in patients with β-TM in the absence of overt heart failure was not exceeding the normal level in our laboratory of 125 pg/ml, but was significantly higher than the control group with a positive correlation with E/Em ratio indicating a stiff myocardium with restrictive diastolic abnormality. It seems in this population, as observed previously, that myocardial disease go through a stage of impaired relaxation before development of systolic dysfunction.[8,9] This finding is in agreement with one report where patients with β-TM with no heart failure had higher NT-pro-BNP and E/Em ratio compared with controls with positive correlation between both variables.[25] In one study on β-TM patients, it was found that NT pro-BNP serum level was significantly increased in patients with documented left ventricular diastolic dysfunction on echocardiogram.[26]

In the β-TM group, the increase ratio of E/Em ratio compared with the control group is highly suggestive of stiff myocardial wall and increased left ventricular end diastolic pressure LVEDP.[27]

Conclusion

We conclude that patients with β-TM had a significantly higher serum level of NT-pro BNP that is positively correlated with the E/Em ratio on tissue Doppler. Furthermore, we confirm our previous findings that patients with β-TM exhibit on echocardiogram a LV diastolic pattern suggestive of restrictive type with well preserved left ventricle systolic function.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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