Determining Nt-proBNP Levels with Diastolic Dysfunction in Thalassemia Major Patients

Mable Misha Singh¹ Ravindra Kumar² Satyendra Tewari³ Sarita Agarwal¹

¹Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

²Department of Genetics, National Institute for Research in Tribal Health, Indian Council of Medical Research, Jabalpur, Madhya Pradesh, India

³Department of Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Address for correspondence Sarita Agarwal, PhD, Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India
(e-mail: saritasgpgi@gmail.com).

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Abstract

Beta thalassemia is an autosomal, recessive disorder, characterized by ineffective erythropoiesis. Chronic transfusions and inability of body to eliminate iron lead to an iron overload, thereby causing damage to heart. Natriuretic peptides (NPs) are produced within the heart, which are then released into the circulation in response to ventricular wall stress. We, therefore, aimed to study the relation between ventricular dysfunction and N-terminal pro-B-type natriuretic peptides (NT-proBNPs). We enrolled 105 patients with increased serum ferritin levels and echocardiography was performed. We collected blood samples and NT-proBNP levels were measured in them. Though we found that the studied group had no significant difference in the mean serum NT-proBNP levels, in patients with or without hypertrophy ($n = 37$, $p = 0.992$), the NT-proBNP levels were found to be significantly increased in patients with diastolic dysfunction ($n = 24$, $p < 0.0001$ with mean values of 577.67 ± 122.01 and 456.50 ± 48.40 pg/mL in patients with and without diastolic dysfunction, respectively). The NT-proBNP levels correlated well with the echocardiography indices, such as left ventricular end-systolic diameter (LVESD), ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/E' ratio), and ratio of the early (E) to late (A) ventricular filling velocities (E/A ratio), and were found to have significant positive correlation with the serum ferritin levels. The NT-proBNP levels correlated significantly with diastolic dysfunction; thus, serum ferritin levels could be useful for assessing the diastolic dysfunction in patients with beta thalassemia.

Keywords

- ▶ NT-proBNP
- ▶ diastolic dysfunction
- ▶ thalassemia major

Introduction

Beta (β)-thalassemia major (β -TM), a hereditary, chronic, hemolytic anemia, caused by more than 200 mutations in human globin gene, manifests itself with a typical lifelong requirement of regular blood transfusion to keep hemoglobin levels close to normal and allow an adequate tissue oxygenation. The chronic administration of large amount of blood

together with extravascular hemolysis and increased iron absorption in the intestinal system lead to iron deposition in the heart, liver, lung, and other organs of the patient with β -TM.¹ Even with the progress in chelation therapy, iron-induced cardiomyopathy still remains a major cause of morbidity and mortality in transfusion dependent β -TM.^{2,3}

Though magnetic resonance imaging (MRI) has gained prominence in the past years, owing to a lack of widespread

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availability, many studies still focus on echocardiography for the evaluation of cardiac function.^{4,5} Some studies favor use of biochemical markers along with echocardiography for a better evaluation of cardiac function.^{6,7} Cardiac natriuretic hormones are synthesized and secreted by mammalian heart, and have potent diuretic, natriuretic, and vascular smooth muscle-relaxing effects; also, they have complex interactions with the hormonal and nervous systems.⁸ B-type natriuretic peptide (BNP) and its amino-terminal cleavage pro-peptide equivalent, N-terminal pro-B-type natriuretic peptide (NT-proBNP), are hormones with natriuretic and vasodilator properties. They are synthesized primarily by cardiomyocytes and are released into the circulation in response to an elevated ventricular wall stress or ischemia. NT-proBNP has a longer half-life than BNP and has more stability and apparently less inpatient variability.⁹ The median day-to-day coefficient of variation for BNP is approximately 25% compared with 20% for NT-pro BNP.^{10,11} NT-proBNP levels also rise more gradually for a given level of cardiac dysfunction. Previous studies also examined the association of NT-proBNP levels with left ventricular diastolic dysfunction in patients with β -TM; however, these studies had a smaller sample size and lacked the significant statistical power to draw any conclusion.^{12–14}

Therefore, the aim of this study was to examine the relationship of serum NT-proBNP levels with diastolic dysfunction in patients with β -TM.

Materials and Methods

Subjects

During the study period, 105 patients with β -TM of age above 10 years were recruited from the Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Uttar Pradesh, India. All patients were on hypertransfusion and iron chelation therapy. Patients with any congenital or acquired heart disease; concurrent infective disorder; end stage renal disease; diabetes mellitus; and with history of any type of cardiac surgery were excluded from the study. Patients on cardio protective drugs were also excluded from the study. The study protocol was approved by the institutional ethics committee and a written informed consent was obtained from all the adult patients or their parents if patient's age was below 18 years.

Echocardiography

Two-dimensional echocardiogram was performed in all patients using GE Vivid 7 by the single author (S.T.). Diastolic dysfunction was determined according to the recommendations of the American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI), 2016.¹⁵

Using the M-mode echocardiography, left ventricular end-diastolic dimension (LVEDd); intra-ventricular septal thickness at diastole (IVSd); and posterior wall thickness diastole (PWTd) were measured at end-diastole. Left ventricular (LV) mass was calculated using the formula: $0.8\{1.04[(LVEDd + IVSd + PWTd)^3]$

$- LVEDd^3]\} + 0.6$.¹⁶ LV mass index (LVMI) was calculated by dividing the LVM (g) by the height (in meters)^{2,7} to correct the left ventricular mass (LVM) for body size.¹⁷ Left ventricular hypertrophy (LVH) was defined using the age and gender specific cutoff values of LVMI.¹⁸

Laboratory Analysis

Peripheral venous blood was collected in a plain tube under aseptic precautions. To avoid the diurnal variations of NT-proBNP, all the samples were collected at the same time (10:00 to 11:00 a.m.). Serum was separated and stored at -80°C until the estimation was done. Serum ferritin and NT-proBNP levels were measured by enzyme-linked immunosorbent assay (ELISA).

Statistical analyses

Data were entered in Microsoft Excel and analyzed on SPSS 20.0 (IBM SPSS, SPSS Inc., United States). Normality of the data was assessed using Kolmogorov-Smirnov (K-S) test; data were found nonparametric for serum NT-proBNP and ferritin levels. Mann-Whitney U test was applied to see the difference in the median serum NT-proBNP levels in patients with or without diastolic dysfunction and patients with or without left ventricular hypertrophy. Spearman's correlation test was performed to identify the correlation between serum NT-proBNP levels and echocardiographic parameters. A receiver operative characteristic (ROC) curve was drawn to find out the cutoff value of NT-proBNP for predicting the diastolic dysfunction. A p -value < 0.05 was considered significant.

Results

Our study group comprised patients with mean age of 16.4 ± 5.1 years. **Table 1** shows that LVH was present in 37(31.4%) patients, of which 21 were males and 16 females. Left ventricular diastolic dysfunction was observed in 24 of the 105 patients, and NT-proBNP levels were significantly ($p < 0.0001$) elevated in patients with diastolic dysfunction (577.67 ± 122.0 pg/mL) than in patients without diastolic dysfunction (456.50 ± 48.40 pg/mL). Mean value of serum NT-proBNP levels in males and females with diastolic dysfunction were 541.9 ± 71.0 and 637.3 ± 166.1 pg/mL, respectively, which were statistically insignificant ($p = 0.138$).

Table 2 shows correlation of NT-proBNP levels with the echocardiography indices. LVESD ($r = 0.275$, $p = 0.014$), E/E' ratio ($r = 0.664$, $p < 0.0001$), and E/A ratio ($r = 0.614$, $p < 0.0001$) were found to be significantly and positively correlated with serum NT-proBNP levels.

Spearman's test revealed a significant correlation between NT-proBNP and serum ferritin levels ($r = 0.306$, $p = 0.006$) (**Fig. 1**). However, there was no significant difference in serum ferritin values in the patients with and without diastolic dysfunction ($p = 0.121$).

The cutoff value as a predictor of diastolic dysfunction for NT-proBNP level was more than 81 pg/mL with a sensitivity of 87.5% and specificity of 85.7% (**Fig. 2**). The area under the curve was 0.868 with a p -value < 0.0001 .

Table 1 Serum NT-proBNP and ferritin levels in patients with and without diastolic dysfunction

Serum NT-proBNP (pg/mL)	Diastolic dysfunction		p-Value
	Present	Absent	
Serum NT-proBNP (pg/mL)	577.6 ± 122.0	456.5 ± 48.4	<0.0001
Serum NT-proBNP in males (pg/mL)	541.9 ± 71.0 ^a	452.91 ± 34.2	<0.0001
Serum NT-proBNP in females (pg/mL)	637.3 ± 166.0 ^a	462.1 ± 64.1	0.001
Serum ferritin (ng/mL)	3681.04 ± 1084	3391.6 ± 1121	0.121

^ap-Value = 0.138.**Table 2** Correlation of NT-proBNP with echocardiography

Echo parameters	Spearman Rho coefficient	p-Value
LVEDD	0.275	0.014
LVEDD	0.157	0.164
SV	−0.005	0.965
EDSV	−0.068	0.548
ESSV	−0.123	0.278
LA	0.131	0.245
Aorta	0.147	0.194
E/E' ratio	0.664	<0.0001
E/A ratio	0.614	<0.0001
LVEF	0.109	0.337

Abbreviations: E, early mitral inflow velocity; E', mitral annular early diastolic velocity; EDSV, end diastolic stroke volume; LA, left atrial diameter; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; SV, stroke volume.

Fig. 1 Scattered dot plot between serum ferritin and serum NT-proBNP levels.

Discussion

The study aimed to evaluate the correlation between NT-proBNP levels and left ventricular diastolic dysfunction in patients with thalassemia major.

Fig. 2 ROC curve for predicting cutoff value of NT-proBNP for diastolic dysfunction in patients with thalassemia. Area under the curve was 0.868 with 95% confidence interval (CI) of 0.769 to 0.968.

The increased ferritin levels are attributed to chronic hemolysis and repeated transfusions.¹⁹ Thalassemia cardiomyopathy is silent at appearance, manifesting as diastolic dysfunction. It occurs as a consequence of iron deposition in the cardiomyocytes. Early diagnosis of cardiac dysfunction is very important for the survival.^{20,21}

Natriuretic peptides are released in response to an increased cardiac volume and pressure overload.²¹ Primarily, these peptides are synthesized as high-molecular-weight precursors; later, they are cleaved into an amino-terminal segment of the precursor molecule (NT-proBNP) and the biologically active peptides.²² Studies show appearance of NT-proBNP in response to wall stress as a sensitive biomarker for the diagnosis, prognosis, and treatment of heart failure.^{23,24} This study was in accordance with the previous studies in patients with β -thalassemia major, illustrating NT-proBNP as a sensitive biomarker for the detection of asymptomatic left ventricular diastolic dysfunction.^{12,13,25–27}

Though serum ferritin levels did not show any significant difference in the patients with and without diastolic dysfunction, the levels correlated well with the increased NT-proBNP levels. However, Alizadeh et al in their study did not find any correlation of NT-proBNP levels with severity of diastolic dysfunction.²⁸ Nikolidakis et al²⁹ and

Delaporta et al³⁰ have reported a significant increase in NT-proBNP levels in cardiac hemosiderosis without any signs of heart failure. Thus, increased serum ferritin levels may be the reason for chronic heart problems leading to diastolic dysfunction in β -thalassemia and release of NT-proBNPs. Our study showed a significant difference in NT-proBNP levels between the patients with and without diastolic dysfunction, with a positive correlation with LVESD and E/A and E/E' ratios, which is also shown by Garadah et al.²⁷ Observations by Kremastinos et al and Dokainish et al report that E/E' velocity correlates well with NT-proBNP levels,³¹ which is in accordance with our study.

Thus, a combination of biochemical markers and changes in echocardiography indices can be used to assess the left ventricular diastolic dysfunction for initiating targeted chelation. Some authors suggest that echocardiography findings may be important in determining the individual target level.³² Also, NT-proBNP seems to be a reliable index for detecting isolated diastolic dysfunction³³ and for early detection of cardiac hemosiderosis.³⁴

There are certain limitations in our study. We did not perform the cardiac MRI for evaluating the cardiac iron load and did not correlate it with NT-proBNP levels.

In conclusion, serum NT-proBNP levels can be used for an early prediction of diastolic dysfunction in patients with thalassemia.

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Conflict of Interest

None.

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