Correlation of N-terminal pro-B-type natriuretic peptide levels and cardiac magnetic resonance imaging T2* in patients with β-thalassaemia major

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Background. Cardiac death secondary to myocardial iron toxicity occurs in 50% of patients with transfusion-dependent β-thalassaemia major. N-terminal pro-B-type natriuretic peptide (NT-proBNP) seems to be a useful tool for early detection of cardiac haemosiderosis. We designed this study to determine whether plasma NT-proBNP levels are predictive of cardiac iron concentration, based on heart T2* assessment by magnetic resonance imaging (MRI).

Materials and methods. We evaluated plasma NT-proBNP levels in 50 patients with β-thalassaemia major, aged 18 to 46 years, with preserved left ventricular systolic function, all of whom had undergone cardiac MRI within 3 months before the study. Next, three groups were defined based on heart T2* value as: group A, patients without evidence of cardiac iron overload (T2*>20 ms); group B, patients with mild to moderate cardiac iron overload (10 ms<T2*<20 ms); group C, patients with severe cardiac iron overload (T2*<10 ms).

Results. NT-proBNP level was not similar among the three groups (p=0.03), being significantly higher in patients in group C (1,104.2±350.5 pg/mL) than in patients in group B (565.9±116.9 pg/mL, p=0.03) or group A (563.5±162.5 pg/mL, p=0.04). The analyses indicate that NT-proBNP levels did not correlate with cardiac iron concentrations (r=0.152, p=0.148).

Discussion. Based on our study, measurements of NT-proBNP levels are not sufficient for early detection of cardiac iron overload. However, NT-proBNP measurements might be used as a tool to guide iron chelation therapy in patients with severe cardiac iron overload. The determination of their clinical use still requires multicentre studies.

Keywords: pro-brain natriuretic peptide, magnetic resonance imaging, beta-thalassaemia, iron overload.

Introduction

Thalassaemias are the most common genetic disorders worldwide[1]. Patients with β-thalassaemia major require regular transfusion therapy to maintain haemoglobin levels of at least 9 to 10 g/dL. Both transfusion therapy and excess gastrointestinal iron absorption lead to iron overload which causes most of the mortality and morbidity associated with thalassaemia. Iron deposition occurs in visceral organs (mainly in the heart, liver, and endocrine glands), causing tissue damage and organ failure[2]. Cardiac death secondary to myocardial iron toxicity occurs in 50% of patients with transfusion-dependent β-thalassaemia major[3]. The onset of heart failure is ominous, often presaging death within a year[1]. Global systolic left ventricular function is usually preserved until severe cardiac toxicity has developed[3], illustrating the importance of having a sensitive method for early detection of iron-overloaded cardiomyopathy.

In this respect, until recently, heart involvement was difficult to assess and cardiac iron was hard to measure. Diagnostic tools such as electrocardiography, 24-hour tracing, and echocardiography, nuclear studies are not predictive of subsequent cardiac dysfunction. When such investigations give positive results, the cardiomyopathy is often advanced[4]. At present, cardiac magnetic resonance imaging (MRI) T2-star (T2*) is the most sensitive investigation of iron deposition. Although the measurement is simple and quick with high reproducibility[4], cardiac MRI T2* is expensive and not easily available, especially in developing countries which have high prevalence of β-thalassaemia major[5,6]. B-type natriuretic peptide (BNP) and its amino-terminal cleavage pro-peptide equivalent, N-terminal pro-B-type natriuretic peptide (NT-proBNP) are released from cardiomyocytes in response to stretching, and highly precise assays are available for their detection in blood[3]. NT-proBNP seems to be a useful tool for early detection of cardiac haemosiderosis[5].

We undertook a cross-sectional study to evaluate plasma NT-proBNP levels in patients with β-thalassaemia

major and to determine whether plasma NT-proBNP levels are predictive of cardiac iron concentration, based on heart T2* assessment by MRI.

Materials and methods

Study population

A convenience sample of 50 adult patients, aged 18 to 46 years, with transfusion-dependent β-thalassaemia major was recruited from the thalassaemia centre in Isfahan (Iran). Patients were eligible for inclusion if they had asymptomatic β-thalassaemia major with a left ventricular ejection fraction >50% and had undergone cardiac MRI in their routine follow-up. The time between the cardiac MRI and sampling had to be less than 3 months. Patients were excluded from this study if they had more than moderate valvular heart disease, a history of coronary artery disease, rhythm disturbances, pericardial disease, acute systematic or chronic pulmonary disease, impaired thyroid, renal, or liver function, or cardiovascular treatment. All patients were receiving regular blood transfusions (mean number of transfusion sessions in the year prior to study entry 20±4.9, mean total amount transfused in the year prior to study entry 234.3±52.7 mL/kg) and chelation therapy, which had been started at least 3 years before the time of sampling (mean duration of chelation therapy 14.5±6.4 years). Twenty-two patients were receiving chelation therapy with deferoxamine, three with deferiprone, four with deferasirox and 21 with a combination of deferoxamine and deferiprone. Splenectomy had been performed in 23 (46%) patients.

Each patient enrolled in this study was thoroughly surveyed by taking a history and performing a physical examination and 12-lead electrocardiography at the time of collecting blood samples. All study participants underwent echocardiography as well as thyroid, renal, and liver function tests and serum ferritin level measurements, based on the routine follow-up protocol for β-thalassaemia patients at the study centre. The study protocol was approved by the Human Research Committee at Medical University of Isfahan and written informed consent was obtained from all patients.

N-terminal pro-B-type natriuretic peptide measurements

All blood samples for the measurement of NT-proBNP were collected just before the patients' scheduled transfusion of packed red blood cells. The patients remained in a supine position for 20 minutes, after which 5-mL samples were collected into clot-tubes and centrifuged at 3,000 rpm for 10 minutes within 30 minutes. Sera were extracted and stored at −80 °C until analysed. NT-proBNP levels were determined by a one-step sandwich enzyme-linked immunosorbent assay (ELISA) using the Novegent NT-proBNP ELISA kit (Chongqing Novegent Biotech Co., Chongqing, China). Clinical chemists were blind to any information regarding the patients' medical records.

Cardiac and liver iron concentration

MRI scans were performed using a 1.5 Tesla magnetic scanner (Symphony, Siemens, Erlangen, Germany). Each scan included measurements of the liver R2 value and the myocardial T2* value, and lasted about 10-15 minutes. For the measurement of heart T2*, a single short axis mid-ventricular slice was acquired during 12-17 seconds of breath-hold (field of view: 298-398 mm; repetition time: 125-223 ms; echo time: 2.6-22.6 ms [8 echo times]; flip angle: 20; slice thickness: 10 mm; matrix: 256 pixels; number of averages: 1; bandwidth: 810 Hz/pixel). All analyses of heart T2* images were performed blinded to the patients' details.

The formula used to determine cardiac iron concentration was:

$$[Fe]=45\times[T2^*]^{-1.22}$$

where [Fe] is measured in milligrams per gram dry weight and T2* is measured in milliseconds[7].

We defined three groups based on the cardiac T2* value: group A, consisting of patients without evidence of cardiac iron overload (T2*>20 ms, [Fe]<1.1 mg/g dry weight); group B, comprising patients with mild to moderate cardiac iron overload (10 ms<T2*<20 ms, 1.1<[Fe]<2.7 mg/g dry weight); and group C, formed of patients with severe cardiac iron overload (T2*<10 ms, [Fe]>2.7 mg/g dry weight)[8].

Statistical analysis

All tests were carried out with SPSS version 16 software. Percentages for nominal or ordinal data and means and standard deviations for continuously scaled variables were calculated. The calculations were performed using Pearson's correlation coefficients, the chi-square test, an independent T-test, and the Mann-Whitney test. Subsequently, one-way analysis of variance (ANOVA) was used to compare means of plasma NT-proBNP levels between the three groups. This was followed by post-hoc least significant difference (LSD) analysis. Receiver operating characteristic analysis was used to assess the predictive value of NT-proBNP concentrations. Statistical significance was set as p-values <0.05.

Results

We studied 50 patients (mean age 28±6.4 years, 42% [n=21] male, 58% [n=29] female) with transfusion-dependent β-thalassaemia major. The patients'

demographic and baseline characteristics are presented in Table I.

According to the analyses, the mean value of heart T2* was 16.9±8.3 ms (range, 6 to 42.7 ms) in our study patients. Of the 50 patients, 16 had no evidence of cardiac iron overload (group A), 23 had mild to moderate cardiac iron overload (group B) and 11 had severe cardiac iron overload (group C). Liver iron concentration ranged from 0.61 to 13.91 mg/g dry weight (mean 6.4±3.6 mg/g dry weight). The results of these analyses are shown in Table II.

The NT-proBNP level ranged from 0.1 to 2,710 pg/mL (mean±SE, 675±106.4 pg/mL) and 41 of 50 (83.7%) patients had a NT-proBNP level higher than the upper limit of normal controls (male <92.6 pg/mL, females <177.6 pg/mL). The NT-proBNP levels differed among the three groups of patients (p=0.03), being significantly higher in group C (1,104.2±350.5 pg/mL) than in group B (565.9±116.9pg/mL, p=0.03) or group A (563.5±162.5 pg/mL, p= 0.04).

The analyses indicate that NT-proBNP levels had no correlation with cardiac iron concentrations (r=0.152, p=0.148). Using receiver operator characteristic analysis, we found that severe cardiac iron overload can be ruled out by a NT-proBNP level at a cut point of 214.5 pg/mL with a sensitivity of 90% and a specificity of 38.5% (area under the curve=0.668). The negative predictive value of this cut point was 93.8%, which means only 1 of 16 patients with NT-proBNP level of less than 214.5 pg/mL was classified as a false-negative. The results of these analyses are shown in Table III.

Based on the analyses, no significant correlation was found between NT-proBNP levels and patients' age (r=0.002, p=0.495) but NT-proBNP was inversely correlated with patients' body mass index (r=−0.243, p=0.046). There was no correlation between NT-proBNP levels and number of transfusion sessions or total amount transfused in the year prior to study entry (p=0.383 and p=0.243, respectively) and

Table I - Demographic and baseline patients' characteristics.

	Group A [T2*>20 ms]	Group B [10 ms<T2*<20 ms]	Group C [T2*<10 ms]
Demographic variables			
Female/male (n)	12/4	13/10	4/7
Age (years)	27.7±5.8	27.7±7.2	28.2±6.1
Body mass index (kg/m^2)	22.2±3.2	21.5±4.2	22.7±7.7
Previous transfusion and chelation therapy			
N. of transfusion sessions in the year prior to study entry	19.3±5.1	19.7±5.1	22.4±3.3
Total amount transfused in the year prior to study entry (mL/kg, mean ± SE)	228.2±13.5	239.2±10.2	233±19.8
Duration of chelation therapy (years)	13.5±7.4	15.4±6.1	14±6

SE: standard error.

Table II - Cardiac and biochemical parameters.

	Group A [T2*>20 ms]	Group B [10 m<T2*<20 ms]	Group C [T2*<10 ms]
Heart T2* (ms)	26.90±6.36	14.05±2.64	8.14±1.37
Cardiac iron concentration (mg/g dry weight)	0.85±0.18	1.87±0.40	3.61±0.76
Liver iron concentration (mg/g dry weight)	4.09±3.69	7.02±2.78	8.61±3.72
Ferritin (ng/mL) (mean ± SE)	2,244.68±584.94	2,715.91±414.05	3,913.36±690.26
NT-proBNP (pg/mL) (mean ± SE)	563.5±162.5	565.5±116.97	1,104.2±350.5

SE: standard error.

Table III - Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) according to different cut-off values.

	Cut-off concentration (pg/mL)	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
NT-proBNP	214.5	90%	38.5%	27.3	93.8	48
	220.5	80%	38.5%	25	88.2	46
	252.5	70%	46.2%	25	85.7	51

no correlation between NT-proBNP levels and duration of chelation therapy (p=0.333). Finally no significant correlation was found between NT-proBNP and ferritin levels (r=0.048, p=0.372) nor between NT-proBNP levels and liver iron concentration (r=0.050, p=0.365).

Ferritin levels were increased and group C patients had higher ferritin levels than those in groups A and B (Table II). There was a significant correlation between ferritin levels and cardiac iron concentration (r=0.245, p=0.043). Ferritin levels also correlated positively with liver iron concentration (r=0.491, p<0.001). A significant correlation between cardiac and liver iron concentrations was also found (r=0.344, p=0.007). It seems that cardiac iron concentrations were higher in male patients (p=0.047).

Discussion

The most important finding of our study was the significant increase of NT-proBNP levels in patients with cardiac T2* values <10 ms (compared to the levels in patients with cardiac T2* values >10 ms). NT-proBNP levels did not differ significantly between patients in groups A and B. We found an inverse correlation between NT-proBNP level and patients' body mass index while no correlation was observed between NT-proBNP levels and patients' age, previous transfusion or chelation therapy, ferritin level, liver iron concentration or cardiac iron concentration.

Heart failure resulting from cardiac iron overload is the leading cause of death in patients with β-thalassaemia[9,10]. Serum ferritin and hepatic iron levels do not reflect cardiac iron overload because of a different transport mechanism from that in the heart, and chelation therapy readily removes iron from the liver. Cardiac MRI T2* is the most sensitive assessment for iron deposition[3]. All patients with a T2*<20 ms develop heart failure secondary to iron overload and T2*<10 ms is even more specific. The predictive value of cardiac T2* for heart failure was assessed by Kirk *et al.*, who found that T2* values <10 ms predicted heart failure with a sensitivity of 97.5% and a specificity of 83%[11]. However, as mentioned above, because of some disadvantages of MRI we tried to determine whether NT-proBNP levels could be used as a first step in detecting cardiac iron overload in patients with transfusion-dependent β-thalassaemia major.

Based on previous studies, β-thalassaemia major is a unique disease characterised by very early diastolic dysfunction, which appears in patients at a young age[12]. BNP and NT-proBNP are biomarkers which increase significantly in documented left ventricular diastolic dysfunction, while NT-proBNP seems to be slightly more sensitive for the detection of latent left ventricular diastolic dysfunction in patients with β-thalassaemia major[13], possibly because of its longer half-life and the fact that it circulates at higher concentrations[14]. Based on one study of 52 β-thalassaemia major patients with normal left ventricular systolic function, NT-proBNP levels increase before Doppler echocardiographic indices become abnormal, suggesting that this biomarker could be suitable for early detection of left ventricular diastolic dysfunction in β-thalassaemia major[15].

Although Tanner *et al.* observed that BNP measurements had very limited value in identifying cardiac haemosiderosis and a significant relation between cardiac T2* and BNP levels was seen only in the few patients with very high values, not in asymptomatic patients[16], a significant association of NT-proBNP levels and cardiac iron concentration was demonstrated, for the first time, in a study of 187 asymptomatic β-thalassaemia major patients by Delaporta *el al.*[5] They observed that NT-proBNP levels were significantly higher in patients with cardiac T2*<20 ms (compared with >20 ms). They also found that correlation of NT-proBNP levels and cardiac iron concentration was significant in patients with cardiac haemosiderosis (T2* values <20 ms), but not in patients without cardiac haemosiderosis (T2*>20 ms)[5]. In our study, we found that NT-proBNP levels were significantly higher in patients with cardiac T2* values <10 ms (compared to T2* values >10 ms), but there was not a significant correlation between NT-proBNP levels and cardiac iron concentration, which is probably because of our small sample size. Since a number of variables affect plasma NT-proBNP levels, including the assay used, age (higher normal values with age), sex (higher values in women), body mass index (lower levels with higher body mass index), and genetic factors[17], another possible reason for the lack of correlation could be demographic and genetic differences.

Contrary to previous studies[5,15], we found no association between NT-proBNP levels and patients' age. Furthermore, in contrast to Kremastinos *et al.*[15], we did not find any correlation between NT-proBNP and serum ferritin. The reason for this difference might be the fact that our measurement was taken at a single time-point, whereas Kremastinos *et al.* evaluated 5-year mean serum ferritin values. There was no meaningful association between NT-proBNP and liver iron concentration in our study, which was also demonstrated in the study by Delaporta *et al.*[5] This may be because NT-proBNP is released predominantly by the ventricles in response to stretching[18].

Another important finding of our study was significant correlations among cardiac iron concentration, liver iron concentration and ferritin levels. Similarly, in a large study of 652 thalassaemia major patients with 1442 MRI scans, there was significant relationship among all three iron variables: cardiac T2*, liver T2* and

ferritin. Cardiac T2* was <10 ms in 98% of patients who developed heart failure and had a greater predictive value than serum ferritin or liver iron concentration[11].

The prevalence of heart dysfunction and/or arrhythmias is significantly higher in males than in females[4]. In the previous observations, cardiac T2* was significantly lower in patients with heart dysfunction, but there was no difference according to sex[19]. We observed that cardiac iron concentration was higher in males, but the relationship was weak.

Limitations

This study has some limitations. First of all, we could not collect samples for NT-proBNP measurements at the same time as the imaging evaluation of patients. This reason as well as our small sample size may have led to the lack of significant correlation between NT-proBNP levels and cardiac iron concentration.

Conclusions

Based on the data collected in this study, measurements of NT-proBNP levels are not sufficient for early detection of cardiac iron overload. Since NT-proBNP levels were significantly increased in patients with severe cardiac iron overload, they might be used as an instrument to guide iron chelation therapy in such patients; however, the determination of their clinical use still requires multicentre studies.

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Authorship contributions

VM and ASK designed the study and supervised data collection. EF collected and analysed data. VM, ASK and EF contributed during article writing and the revision process.

The Authors declare no conflicts of interest.

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