

## Research Article



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# NT-proBNP levels in $\beta$ -thalassemia major patients without cardiac hemosiderosis

## Kardiyak Hemosiderozis Bulunmayan $\beta$ -Talasemi Major Hastalarında NT-proBNP Düzeyleri

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### Abstract

**Introduction:** Heart failure due to hemosiderosis is frequent in beta-thalassemia major ( $\beta$ -TM) patients. Magnetic resonance imaging (MRI) is used in the early detection of heart failure. Amino-terminal pro-brain natriuretic peptide (NT-proBNP) is a very sensitive marker in the diagnosis of heart failure. In this study, we aimed to investigate the efficacy of NT-proBNP levels in thalassemia patients, who are thought to have no cardiac iron deposition according to T2\* scoring system (CMRT2\* > 20 msn), in early identification of cardiac failure.

**Methods:** NT-proBNP levels of 31 patients, who have T2\* > 20 ms, and of 25 healthy population were measured by chemoluminescence method.

**Results:** NT-proBNP levels were not different in thalassemic patients [median: 33 (IQR: 28–94) pg/mL] compared to control group [median: 41 (IQR: 28–59) pg/mL]. We found that NT-proBNP level was above cut-off value in six patients.

**Conclusion:** NT-proBNP is a cheaper, reachable, and noninvasive method compared to MRI technique, it can be easily used in monthly controls. Detection of high

NT-proBNP levels above cut-off values in patients whose T2\* values are normal indicates that measurement of NT-proBNP is a more sensitive marker in early detection of cardiac failure.

**Keywords:** Beta-thalassemia major; Heart failure; NT-proBNP; Cut-off values; Early detection.

### Özet

**Giriş:** Beta-talasemi major ( $\beta$ -TM) hastalarında hemosiderozise bağlı kalp yetmezliği sık görülmektedir. Kalp yetmezliğini erken dönemde tespit etmek için manyetik rezonans görüntüleme (MRI) yöntemi kullanılmaktadır. N-terminal beyin natriüretik peptid (NT-proBNP) kalp yetmezliğinin tanısında oldukça hassas bir belirteçtir. Bu çalışmamızda T2\* skoruna göre kardiyak demir birikimi olmayan (CMRT2\* > 20 msn)  $\beta$ -TM hastalarında, kalp yetmezliğinin erken dönemde tanısında NT-proBNP'nin etkinliğini araştırdık.

**Yöntem:** T2\* > 20 ms olan 31  $\beta$ -TM hastası ve 25 sağlıklı kişide NT-ProBNP düzeyleri kemilüminesans yöntem ile ölçüldü.

**Bulgular:** Hastaların NT-proBNP düzeyleri [ortanca: 33 (IQR: 28–94) pg/mL] ile kontrol grubu [ortanca: 41 (IQR: 28–59) pg/mL] arasında anlamlı bir fark tespit edilmedi. Hastaların 6'sında NT-proBNP düzeylerinin cut-off değerinin üzerinde olduğu belirlendi.

**Sonuç:** MRI yöntemi ile kıyaslandığında NT-proBNP daha ucuz, ulaşılabilir ve girişimsel olmayan bir yöntemdir. Talasemi hastalarının aylık kontrollerinde daha kolaylıkla uygulanabilir. T2\* skoru normal olan hastaların bazlarında NT-proBNP düzeylerinin cut-off değerlerinden daha yüksek bulunması, NT-proBNP'nin kalp yetmezliğini erken dönemde tespit etmede daha hassas bir belirteç olduğunu göstermektedir.

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**Anahtar Kelimeler:** Beta-talasemi major; Kalp yetmezliği; NT-proBNP; Cut-off değerleri; Erken Teşhis.

(CMRT2\* $>20$  ms), in early identification of cardiac failure.

## Introduction

Patients with beta-thalassemia major ( $\beta$ -TM) are maintained on continuous blood transfusion regimens to keep hemoglobin levels close to normal and allow adequate tissue oxygenation. The chronic administration of large amounts of blood, combined with extravascular hemolysis and an increase in the intestinal absorption of iron, inevitably leads to significant hemosiderosis of all organs, including the heart [1]. Hemochromatosis alone or in combination with immunogenetic factors is the main pathogenetic mechanism of heart failure [2]. Although iron chelation therapy has markedly improved outcomes, cardiac complications remain the major cause of death in patients with transfusion-dependent  $\beta$ -TM [3]. Heart failure in these patients, which may be reversible but has a poor prognosis, is characterized by myocardial iron deposition-related early diastolic dysfunction [4]. Therefore early detection of heart failure is very important in thalassemia patients [5]. Cardiac magnetic resonance T2\* (CMRT2\*) represents the only imaging method in clinical use with the potential to detect iron within the heart. Clinically important iron loading is defined by T2\* values of  $<20$  ms and severe cardiac iron loading is considered to be present if cardiac T2\* is  $<10$  ms [6]. However, CMRT2\* is a relatively expensive technique and it is not widely available especially in the developing countries [7]. The level of amino-terminal pro-brain natriuretic peptide (NT-proBNP), the biologically inactive N-terminal fragment of the active hormone BNP, predicts cardiac mortality and morbidity in the general population as well as in cohorts of patients with heart failure and stable coronary heart disease. Small increases in circulating NT-proBNP are associated with an increased risk of cardiovascular events, and high levels signal left ventricular dysfunction [8]. The associations between clinical and laboratory markers of  $\beta$ -TM and levels of NT-proBNP have been examined. Increase in the level of NT-proBNP in patients with cardiac hemosiderosis was detected in previous studies. But there are a few studies related to NT-proBNP levels in thalassemia patients without cardiac hemosiderosis [9]. In this study, we aimed to investigate the efficacy of NT-proBNP levels in thalassemia patients, who are thought to have no cardiac iron deposition according to T2\* scoring system

## Materials and methods

### Patients population

Thirty-one patients [median: 23 (IQR: 18–27) years] who were diagnosed as  $\beta$ -TM in the thalassemia center of Antalya Education and Research Hospital, were enrolled in this study. Cardiac iron load was measured by MRI. The patients whose CMRT2\* $>20$  ms [median: 30.1 (IQR: 24.6–44.1) ms] were included into the study. All  $\beta$ -TM patients without symptoms or signs of HF were included in the study. The presence of valvular heart disease, history of coronary artery disease, previous systolic dysfunction, rhythm disturbances, pericardial disease, acute systematic or pulmonary disease and impaired thyroid, renal, liver function or cardiovascular treatment were exclusion criteria for this study. All patients were receiving regular blood transfusion every 2–4 weeks and regular chelation therapy. Twenty-five healthy, age-matched volunteers [median: 26 (IQR: 19–30) years] with no evidence of cardiovascular disease were selected for the control group. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all patients.

All blood samples for the measurement of NT-proBNP were withdrawn at the same day following transfusion. All blood samples obtained from patients and controls were collected in EDTA tubes. All samples were centrifuged at 3000 rpm for 10 min. Plasma samples were stored at  $-80^{\circ}\text{C}$ . NT-proBNP was measured by chemoluminescence immunoassay method in Siemens Immulite 2000 instrument, as pg/mL. NT-proBNP levels of patients were evaluated according to both control group values and cut-off values of manufacturer ( $<110$  pg/mL, for age below 75). Serum ferritin levels were measured by chemoluminescence immunoassay method in Beckmann Coulter DXI instrument, as ng/mL.

### Statistical analysis

Statistical analysis of clinical data between two groups (control vs. patients) were carried out using T-test and Mann Whitney U-test. Analyses were performed with PASW 18 (SPSS/IBM, Chicago, IL, USA) software and

two-tailed p-value  $<0.05$  was considered statistically significant.

## Results

The study included 31 patients with transfusion-dependent  $\beta$ -TM. None of the patients had evidence of cardiac siderosis, defined as  $\text{Fe} < 1.1 \text{ mg/g}$  dry weight, corresponding to  $\text{T2}^* > 20 \text{ ms}$ . Minimal atrial dilatation was detected in four patients in echocardiography. Clinical and laboratory findings of the study population are given in Table 1. Patients [median: 23 (IQR: 18–27) years, 14 male, 17 female] and controls [median: 26 (IQR: 19–30) years, 14 male, 17 female] were well matched for age and sex. Hemoglobin levels were significantly lower in the patients ( $8.87 \pm 0.9 \text{ g/dL}$ ) than in the control group ( $13.91 \pm 1.41 \text{ g/dL}$ ,  $p < 0.05$ ). The median ferritin level of the patients was 2460 (IQR: 1036–5154) ng/mL.

NT-proBNP levels were not different in thalassemic patients [median 33 (IQR 28–94) pg/mL] compared to control group [median: 41 (IQR: 28–59) pg/mL] (Table 1). But when NT-proBNP levels of patients were evaluated according to manufacturer's reference values ( $< 110 \text{ pg/mL}$ ), we found that NT-proBNP level was above reference value in six patients (130, 131, 141, 163, 195, and 328 pg/mL). NT-proBNP levels were above 110 pg/mL in three of four patients who have atrial dilatation in echocardiography (131, 165, 328 pg/mL), and it was below 100 pg/mL in one patient (65 pg/mL). We found a cut-off point of 110 pg/mL

**Table 1:** Clinical, hematological and biochemical characteristics of  $\beta$ -TM patients.

Variable	Controls (n=25)	Patients (n=31)
Age [(median (IQR)]	26 (19–30)	23 (18–27)
Male/female	12/13	14/17
Hb (g/dL) (mean $\pm$ SD)	13.91 $\pm$ 1.41	8.87 $\pm$ 0.9 <sup>a</sup>
Htc (%) (mean $\pm$ SD)	41.22 $\pm$ 3.9	25.79 $\pm$ 2.61 <sup>a</sup>
MCV (fL) (mean $\pm$ SD)	87.12 $\pm$ 2.37	81.25 $\pm$ 2.53 <sup>a</sup>
Ferritin (ng/mL) [median (IQR)]	–	2460 (1036–5154)
T2* (ms) [median (IQR)]	–	30.1(24.6–44.1)
NT-ProBNP (pg/mL) [median (IQR)]	41 (28–59)	33 (28–94)

Values are either median and interquartile range (IQR), frequency or mean and standard deviation (SD). <sup>a</sup>p < 0.05.

with a sensitivity of 75%, a specificity of 88%, a positive predictive value (PPV) of 50%, and a negative predictive value (NPV) of 96% for NT-proBNP. Analytic performance parameters according to different cut-off values are shown in Table 2.

## Discussion

Iron-induced cardiotoxicity is the commonest cause of morbidity and mortality in patients with transfusion-dependent  $\beta$ -TM [10]. Heart failure in these patients, which may be reversible but has a poor prognosis, is characterized by myocardial iron deposition-related early diastolic dysfunction. Early diagnosis of myocardial dysfunction is very important since survival of  $\beta$ -TM patients with heart failure is dramatically improved by intensification of blood transfusions and iron chelation therapy [11].

Quantifying myocardial iron content has only recently become possible by using MRI [12]. Cardiac T2\* MRI identifies patients at high risk of heart failure and arrhythmia from myocardial siderosis in thalassemia major and is superior to serum ferritin and liver iron. Using cardiac T2\* for the early identification and treatment of patients at risk is a logical means of reducing the high burden of cardiac mortality in myocardial siderosis [6]. Kremastinos et al. [5] showed that cardiac T2\* is highly predictive over 1 year for the development of heart failure and arrhythmia, and is significantly more predictive than contemporaneous measures of liver iron and serum ferritin. A widespread program using cardiac T2\* in  $\beta$ -TM has considerable potential to reduce mortality from heart failure by the early identification and treatment of patients at risk [13].

The level of NT-proBNP, the biologically inactive N-terminal fragment of the active hormone BNP, predicts cardiac mortality and morbidity in the general population as well as in cohorts of patients with heart failure and stable coronary heart disease. NT-proBNP has a longer half-life than the active hormone and is a viable

**Table 2:** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) according to different cut-off values for NT-proBNP.

NT-proBNP (pg/mL)	Sensitivity	Specificity	PPV	NPV
110	75%	88%	50%	96%
140	50%	96%	66%	92%
214	25%	100%	100%	90%
300	25%	100%	100%	90%

biomarker of cardiovascular disease. Small increases in circulating NT-proBNP are associated with an increased risk of cardiovascular events, and high levels signal left ventricular dysfunction [14].

Previous studies dealing with natriuretic peptides in  $\beta$ -TM, found that NT-proBNP is a sensitive biomarker for the detection of diastolic and systolic left ventricular dysfunction [15]. Delaporta et al. [16] found that NT-proBNP level was significantly higher in patients with cardiac hemosiderosis compared to patients without cardiac hemosiderosis in thalassemia. Balkan et al. [17] showed that NT-proBNP levels were significantly higher in the patients than controls in their study in which myocardial function was evaluated by doppler-echocardiography. Measurement of  $T2^* < 20$  ms indicates that there is no accumulation of iron in the heart in many studies [18].

Our study was performed in 31  $\beta$ -TM patients who have no cardiac hemosiderosis ( $T2^* > 20$  ms). There was an atrial dilatation in four patients in echocardiography. We found that NT-proBNP levels of patients were not statistically different from that of healthy population. But NT-proBNP levels of 20% of patients had a reference value which is above manufacturer's recommendations (NT-proBNP level at a cut point of 110 pg/mL with a sensitivity of 75%, a specificity of 88%, a PPV of 50% and a NPV of 96%). We found that NT-proBNP level was above reference value in six patients. There are different reference values present for NT-proBNP varying according to age and sex in the literature. Mayo clinic accepts reference values of 10–52 pg/mL and 10–140 pg/mL in males and females ( $\leq 46$  age), respectively. The reference values increase as the age increases [19]. Age of patients ranged between 16 and 37 in our study. According to NT-proBNP level at a cut point of 140 pg/mL, we found a sensitivity of 50%, a specificity of 96%, a PPV of 66% and a NPV of 92%. Mehrzad et al. [20] reported a sensitivity of 90% and a specificity of 38.5% in a study in which a cut-off level of 214.5 pg/mL was used. In our study we found a sensitivity of 25% and a specificity of 100% for a cut-off value of 214 pg/mL. It is stated that acute heart failure is not present when NT-proBNP level is below 300 pg/mL (have a 98% NPV) in the literature [19]. We found that NPV was 90% at NT-proBNP cut-off level of 300 pg/mL. We preferred cut-off value of 110 pg/mL at which the highest sensitivity value was present since our aim was to detect cardiac complications at an early period in patients with hemoglobinopathy in our study.

We think that detection of high NT-proBNP levels above cut-off level in 20% of patients, without cardiac iron deposition ( $T2^* > 20$  ms) is an important finding. MRI is a widely used and effective method to diagnose cardiac

complications in thalassemia patients [21]. But  $T2^*$  measurement by MRI is both expensive and unavailable in many centers.  $T2^*$  measurement is recommended one in a year. We think that since measurement of NT-proBNP is a cheaper, reachable, and non-invasive method compared to MRI technique, it can be easily used in monthly controls. Detection of high NT-proBNP levels above cut-off values in patients whose  $T2^*$  values are normal indicates that measurement of NT-pro-BNP is a more sensitive marker in early detection of cardiac failure.

## Conclusions

Measurement of NT-pro-BNP is extremely cheaper, less invasive, and frequently measurable marker in diagnosis and follow up of cardiac complications in thalassemia patients. We think that routine measurement of NT-pro-BNP and  $T2^*$  scoring in patients who have an increasing trend of NT-pro-BNP will be helpful to identify cardiac damage in early period in thalassemia patients.

**Conflict of interest statement:** There are no conflicts of interest among the authors.

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