Acta Cardiol Sin 2016;32:231–238
Original Article
doi: 10.6515/ACS20150824A

General Cardiology

The Association between Serum Ferritin Level, Tissue Doppler Echocardiography, Cardiac T2* MRI, and Heart Rate Recovery in Patients with Beta Thalassemia Major

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Background: It is generally well-understood that iron-mediated cardiomyopathy is the major complication that can arise from beta thalassemia major (TM). Therefore, early diagnosis, risk stratification, and the effective treatment of beta TM patients are clinically important to optimize long-term positive outcomes.

Methods: This study included 57 beta TM patients with a mean age of 25 ± 7 years. We determined the serum ferritin level, echocardiography, heart rate recovery (HRR), and cardiac magnetic resonance (CMR) T2* in all patients. CMR T2* findings were categorized as normal myocardium (T2* > 20 ms), and myocardial involvement (T2* ≤ 20 ms). HRR values at 1-5 min (HRR1-5) were recorded; Subsequently. HRR was calculated by subtracting the heart rate at each time point from the heart rate at peak exercise.

Results: There was a significant negative correlation between the serum ferritin level and the cardiac T2* MRI findings (r = -0.34, p = 0.009). A similar result was found in the negative correlation between serum ferritin and all heart rate recovery values. There was a significant positive correlation between HRR1, HRR2, and HRR3 values, and CMR T2* (T2* heart rate recovery (HRR)1: r = 0.51, p < 0.001; T2* HRR2: r = 0.48, p < 0.001; T2* HRR3: r = 0.47, p < 0.001, respectively).

Conclusions: The serum ferritin level and echocardiography can be used to predict the presence of myocardial iron load in beta TM patients. Therefore, HRR can be used to screen beta TM patients, and the clinical use of HRR can be a predictive marker for autonomic dysfunction in beta TM patients.

Key Words: Beta thalassemia major • Cardiac magnetic resonance T2* • Heart rate recovery • Iron overload • Serum ferritin level • Tissue Doppler imaging

INTRODUCTION

Beta thalassemia major (TM) is a hereditary anemia that develops due to impairment of beta globulin chain synthesis. The prevalence of beta TM is high in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and Thailand.[1] Beta TM patients require regular blood transfusion beginning in infancy because of severe hemolytic anemia. Ultimately, complications in many organs occur due to iron overload, but the major cause of mortality in beta TM patients is myocardial siderosis.

Bone marrow transplantation is an effective treatment method in some beta TM patients, but blood

Received: April 19, 2015 Accepted: August 24, 2015
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transfusion and iron chelation remain the primary therapeutic methods. Iron chelation agents are useful, but iron overload cardiomyopathy can only be reversed if intensive chelation is initiated in early periods.[2,3] Therefore, early recognition of iron overload in beta TM patients is vitally important, and some methods for doing so have been reported by clinical trials and animal studies. The present study aimed to determine among the serum ferritin level, tissue Doppler imaging (TDI) echocardiography, cardiac magnetic resonance T2* (CMR T2*), and heart rate recovery (HRR) in patients with beta TM.

MATERIALS AND METHODS

The study included 57 beta TM patients that were treated at the thalassemia unit of our hospital between January 2014 and December 2014. Patients < 18 years of age, diabetes mellitus or hypertension, prior diagnosis of ischemic heart disease, and patients with systolic dysfunction based on echocardiography were excluded. The serum ferritin level was measured using the standard enzyme-linked immunosorbent assay method 1 week after blood transfusion, and mean values were calculated. Associations between the serum ferritin level and cardiac functions were evaluated according to the ferritin level (< 1000 ng/mL, 1000-2000 ng/ mL, and > 2000 ng/mL). Written informed consent was obtained from every patient and the study protocol was approved by the hospital's ethics committee.

Echocardiographic evaluations were performed for each patient using conventional and tissue Doppler echocardiography. An echocardiography machine (Vivid S5, GE Medical Systems, Horten, Norway) fitted with a 2-4 MHz phased array transducer was used. Echocardiography was performed by a single expert cardiologist blinded to the patients' CMR T2*, HRR, and serum ferritin findings. Tissue Doppler imaging was performed in the apical views to acquire mitral annular velocities. For this imaging, the sample volume is positioned at or 1 cm within the septal and lateral insertion sites of the mitral leaflets and adjusted as necessary (usually 5-10 mm) to cover the longitudinal excursion of the mitral annulus in both systole and diastole. Diastolic dysfunction was considered to be early diastolic (Em)/late diastolic wave (Am) rate of < 1 and, also < 8 cm/sec of septal Em or < 10 cm/sec of lateral Em by TDI. All data were obtained according to the recommendations of the American Society of Echocardiography.[4,5]

Cardiac magnetic resonance imaging (MRI) is the best method for detecting myocardial iron deposition. MRI is non-invasive, readily available, and is reproducibly quantified using myocardial T2*.[6] Furthermore, T2* is a unique magnetic resonance parameter measured in milliseconds that decreases with the level of iron in the heart.[7,8] Myocardial T2* was calculated based on the decay curve generated from a multi-echo gradient echo sequence with a range of echo times, as previously described.[9] The use of the single breath-hold, multiecho acquisition allowed reliable quantification of myocardial T2*. The good reproducibility, speed, and T1 independence of this technique allows for greater accuracy, faster patient throughput, and, therefore reduced costs.[9] MRI was performed within 1 to 2 weeks after blood transfusion. All T2* cardiovascular MRI examinations were performed in the radiology department; all measurements were obtained at 1.5T scanner by experienced technicians using a mid-ventricular short axis slice with a region of interest in the septum.[10] Myocardial T2* > 20 ms was considered normal myocardial iron load, T2* $\leq$ 20 ms was considered myocardial iron load, and T2* $\leq$ 10 ms was considered severe myocardial iron load.[11]

Patients relaxed for nearly 10 minutes in the supine position for heart rate stabilization prior to exercising. In addition, the patients were instructed not to use any substances that could affect heart rate, such as tobacco or caffeine. Symptom-limited treadmill exercise testing was performed on each patient using the standard Bruce protocol. HRR values at the 1st (HRR1), 2nd (HRR2), 3rd (HRR3), 4th (HRR4), and 5th (HRR5) min were recorded. HRR was calculated by subtracting the heart rate at each time point from the heart rate at peak exercise (for example, HRR1 = peak heart rate at exercise-recovery first minute heart rate). Blood pressure and heart rate measurements were obtained during each stage of the exercise test.

Statistical analysis

In this study, data were expressed as mean $\pm$ standard deviation for continuous variables, counts and per-

centages for categorical variables. Data were tested for normal distribution using the Kolmogorov-Smirnov test, and frequencies, means and standard deviations were calculated by descriptive statistics. Regarding the categoric variables, group differences were examined using the Chi-square test. Correlations of variables were evaluated using the Pearson or Spearman's correlation analysis. Values between 0-0.3 indicated a weak correlation, 0.3-0.7 indicated intermediate, and 0.7-1.0 indicated a strong correlation. Multiple linear regression models including ferritin, HRR1, HRR2, and HRR3 were used for predicting T2*after adjusting for other confounders such as demographic and laboratory parameters. A p value < 0.05 was considered statistically significant. Statistical analyses were conducted with a commercially available software package (SPSS version 16.0, SPSS, Chicago, IL, USA).

RESULTS

The study included 57 patients [26 (45.6%) female and 31 (54.4) male] with a mean age of 24.5 ± 6.8 (range: 18-48 years).

All the patients had normal left ventricular (LV) systolic function. However, LV diastolic function did not different significantly between genders (p > 0.05). The patients' primary clinical characteristics and findings are summarized in Table 1. Diastolic dysfunction based on TDI was noted in 8 male and 8 female patients, with an ejection fraction > 55%. There was a negative correlation between the serum ferritin level and lateral Em values by TDI (r = -0.39, p = 0.04). Besides, there was a significant negative correlation between the serum ferritin level and Em/Am ratio (r = -045, p = 0.003). It was also noted that Cardiac T2* was negatively associated with diastolic dysfunction (r = -0.29, p = 0.03).

The median serum ferritin level was 2072 ng/mL (range: 769-4120 ng/mL). The relationship between the serum ferritin level and cardiac T2* MRI is shown in Table 2. There was a significant negative correlation between the serum ferritin level and the cardiac T2* level (r = -0.34, p = 0.009) (Figure 1). There was also a significant negative correlation between the serum ferritin level, and all HRR values. There was a weak correlation between the serum ferritin level and HRR1 (r = -0.31, p = 0.02), a moderate correlation between the serum ferritin level and HRR2 (r = -0.42, p = 0.001), a weak correlation between the serum ferritin level and HRR3 (r = -0.39, p = 0.003), a weak correlation between the serum ferritin level and HRR4 (r = -0.33, p = 0.011), and a weak correlation between the serum ferritin level HRR5 (r = -0.310, p = 0.019). On the other hand, there was not a significant correlation between the serum ferritin level and age (p = 0.053).

The mean cardiac T2* value was 27.58 ± 13.88 ms (8.7-56.4) for all 57 patients. CMR T2* < 20 ms was observed in 20 patients (35%), versus CMR T2* > 20 ms in 37 patients (65%). Table 2 shows the association between CMR T2* and the serum ferritin level. The frequency of abnormal CMR T2* was 80% (n = 16) in pa-

Table 1. The patients' primary clinical characteristics and findings

Characteristics	Patients (n = 57)
Female, n (%)	26 (45.6)
Mean age (range), years	24 (18-48)
Mean (range) serum ferritin level ng/mL	2072 (769-4120)
Cardiac T2*, ms (mean ± SD)	27.58 ± 13.88
T2* category, n (%)	
Abnormal T2* (< 20 ms)	20 (35)
Normal T2* (> 20 ms)	37 (65)
Diastolic dysfunction based on TDI, n (%)	16 (28)
Exercise characteristics	
Exercise capacity (METs) (mean ± SD)	10.1 ± 1.8
Exercise duration in min (mean ± SD)	8.4 ± 1.7
HR reserve (mean ± SD)	107 ± 11
Peak HR (mean ± SD), bpm	175 ± 15
HRR1 (mean ± SD), bpm	29 ± 10
HRR2 (mean ± SD), bpm	48 ± 13
HRR3 (mean ± SD), bpm	54 ± 13
HRR4 (mean ± SD), bpm	61 ± 12
HRR5 (mean ± SD), bpm	62 ± 13

HR, heart rate; HRR, heart rate recovery; HR reserve (bpm), 220-age-HR baseline; METs, metabolic equivalents; TDI, Tissue Doppler imaging.

Table 2. Serum ferritin levels according to T2* MRI findings

	Ferritin level (ng/mL)			Total
	< 1000	1000-2000	> 2000	
Normal T2* MRI	16 (43.2%)	8 (21.7%)	13 (35.1%)	37 (100%)
Abnormal T2* MRI	2 (10%)	2 (10%)	16 (80%)	20 (100%)
Total	18	10	29	57

MRI, magnetic resonance imaging.

tients with a serum ferritin level > 2000 ng/mL. In addition, 29 patients had a serum ferritin level > 2000 ng/mL, of which 35.1% (n = 13) had normal cardiac T2*, whereas 10% (n = 2) of patients with a serum ferritin level 1000-2000 ng/mL had abnormal cardiac T2*. In all, 10 patients had a serum ferritin level of 1000-2000 ng/mL, of which 2 had abnormal CMR T2*, and 2 patients with a serum ferritin level < 1000 ng/mL had an abnormal CMR T2* (Table 2). Based on CMR T2*, the frequency of cardiac siderosis was similar in the male and female patients: 11 (19.3%) males and 9 (15.8%) females. Figure 1 shows the correlation between cardiac T2* and the serum ferritin level.

Table 1 shows the exercise characteristics of the patients. The mean exercise capacity of the patients was 10.1 ± 1.8 metabolic equivalents (METs), mean exercise duration was 8.4 ± 1.7 min, and heart rate reserve (220-age-HR baseline) was 107 ± 11 bpm. There was a significant negative correlation between the serum ferritin level and all HRR values. Additionally, there was a positive correlation between HRR1, HRR2, and HRR3 values, and CMR T2* levels in MRI (T2*-HRR1: r = 0.51, p < 0.001; T2*-HRR2: r = 0.48, p < 0.001. T2*-HRR3: r = 0.47, p < 0.001) (Figures 2-4). On the other hand, there was no correlation between HRR4 or HRR5, and cardiac T2* (p = 0.23 and p = 0.19, respectively).

Multivariable regression analysis was performed between Heart Rate Recovery (HRR 1, 2, 3, 4, 5) data and clinical variables including age, sex, body mass index (BMI), and other confounders. As a result of this analysis, HRR 1, 2, and 3 are only to be associated with the T2* value, and was found to be independent of the other parameters (Table 3).

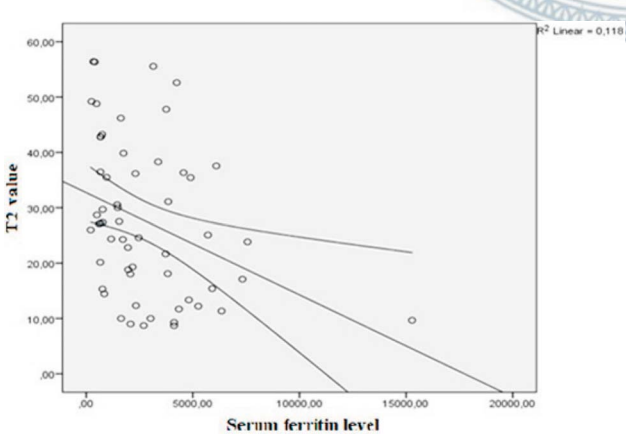

Figure 1. *The correlation between cardiac T2* and the serum ferritin level in beta TM patients. Cardiac T2* was negatively associated with the serum ferritin level (r = -0.34, p = 0.009).*

DISCUSSION

CMR T2* is a commonly used noninvasive method for detecting iron overload in the heart[12]. The CMR T2* option has a high sensitivity and is a repeatable method for evaluating cardiac iron status. It was shown to be correlated not only with systolic function, but also with diastolic function.[12] A high myocardial T2* value is associated with good cardiac functions.[13] There is a negative correlation between myocardial iron deposition and CMR T2* values. Most recent data support the proposi-

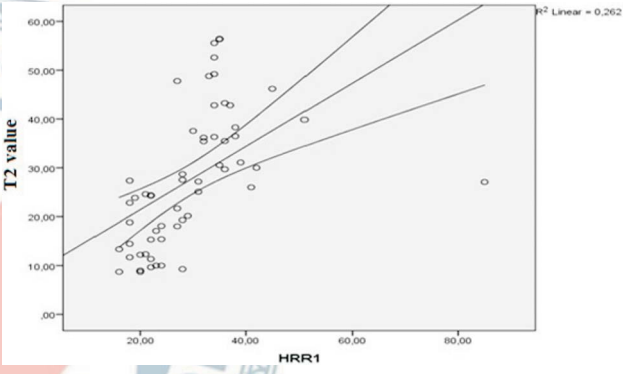

Figure 2. *The correlation between cardiac T2* and HRR1 in beta TM patients. Cardiac T2* was positively associated with HRR1 (r = 0.51, p < 0.001).*

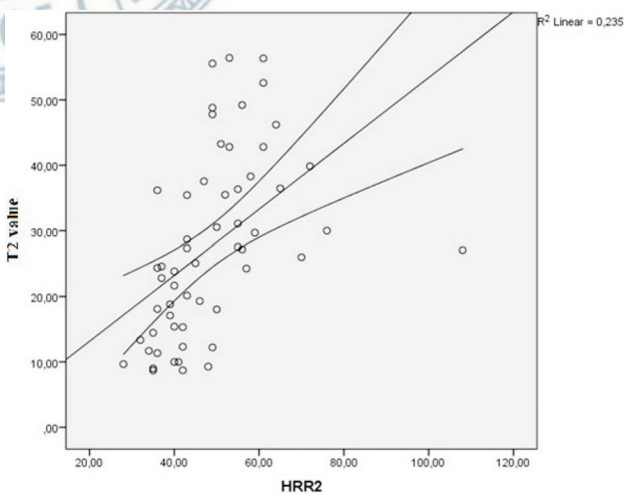

Figure 3. *The correlation between cardiac T2* and HRR2 in beta TM patients. Cardiac T2* was positively associated with HRR2 (r = 0.48, p < 0.001).*

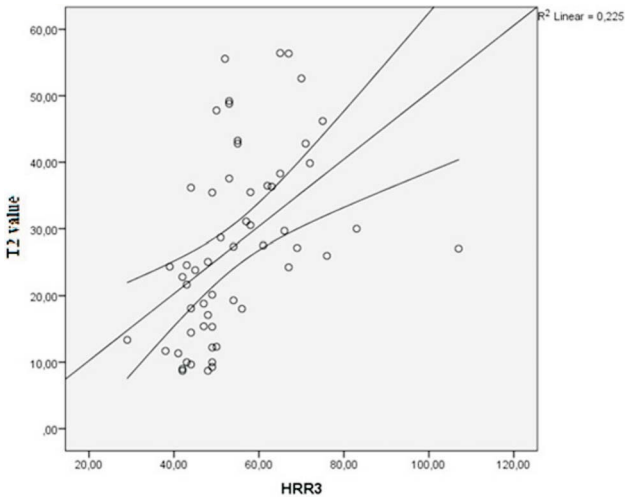

Figure 4. *The correlation between cardiac T2* and HRR3 in beta TM patients. Cardiac T2* was positively associated with HRR3 (r = 0.47, p < 0.001).*

tion that CMR T2* is more specific than previously described methods for determining cardiac iron status. Moreover, CMR T2* can be used for early detection of the cardiac iron level before the onset of symptoms of iron overload cardiomyopathy. However, CMR T2* is a relatively expensive method which requires expert interpretation, and not widely available in many hospitals; as such, other methods are also used for the early detection of cardiac involvement. Therefore, the present study evaluated the association between the CMR T2* value and the serum ferritin level, echocardiography, and HRR values during exercise testing, based on CMR T2* as the reference.

An earlier study that evaluated the risk heart failure by cardiac T2* in patients with beta TM reported that heart failure developed in 98% of those patients with a cardiac T2* < 10 ms. Moreover, a cut-off value of 10 ms

Table 3. Multiple linear regression analyses for predicting T2*

	Variables	Beta	95% Confidence Interval	p value
1st model includes ferritin and other covariates	*Ferritin*	*-0.28*	*-0.003 – 0.00*	*0.03*
	Age	0.14	-0.27 – 0.83	0.32
	Gender	-0.10	-10.69 – 5.07	0.48
	BMI	0.31	0.14 – 3.09	0.03
	Hemoglobin	-0.05	-4.23 – 2.98	0.73
	Glucose	-0.29	-0.16 – -0.01	0.03
	Uric acid	0.23	-0.46 – 3.39	0.13
2nd model includes HRR1 and other covariates	*HRR1*	*0.43*	*0.26 – 0.83*	*0.00*
	Age	0.20	-0.08 – 0.89	0.10
	Gender	0.01	-6.91 – 7.27	0.96
	BMI	0.25	-0.08 – 2.63	0.06
	Hemoglobin	-0.08	-4.27 – 2.08	0.49
	Glucose	-0.26	-0.15 – -0.01	0.04
	Uric acid	0.11	-1.03 – 2.45	0.42
3rd model includes HRR2 and other covariates	*HRR2*	*0.41*	*0.19 – 0.68*	*0.001*
	Age	0.15	-0.21 – 0.80	0.25
	Gender	-0.01	-7.59 – 6.86	0.92
	BMI	0.28	0.10 – 2.85	0.04
	Hemoglobin	-0.04	-3.77 – 2.83	0.78
	Glucose	-0.29	-0.16 – -0.01	0.02
	Uric acid	0.14	-0.83 – 2.70	0.29
4th model includes HRR3 and other covariates	*HRR3*	*0.38*	*0.15 – 0.67*	*0.003*
	Age	0.13	-0.26 – 0.78	0.33
	Gender	0.01	-7.17 – 7.65	0.95
	BMI	0.28	0.04 – 2.85	0.04
	Hemoglobin	-0.05	-4.05 – 2.65	0.68
	Glucose	-0.27	-0.15 – -0.004	0.04
	Uric acid	0.14	-0.89 – 2.72	0.31

* Dependent variable: T2* value. BMI, body mass index; HRR, heart rate recovery.

predicted heart failure with 97.5% sensitivity and 85.3% specificity.[14] Based on these findings, a myocardial T2* level < 10 ms strongly indicates severe iron overload and an elevated risk of heart failure. In the present study beta TM patients with a T2* ≤ 20 ms had more severe diastolic dysfunction based on echocardiography, and higher serum ferritin levels and lower HRR values.

The serum ferritin level has been used as a predictor of iron overload.[15] A serum ferritin level > 1800 μg/L is associated with an elevated cardiac iron concentration, and a level > 2500 μg/L is associated with an increase in the prevalence of cardiac events.[16] However, the serum ferritin level is not specific for iron overload, and may increase in many clinical conditions, including inflammation, liver disease, collagen tissue disease, and malignancy.[17] Moreover, a low serum ferritin level is not associated with a reduction in the risk of iron-induced cardiomyopathy.[6] Many contemporary studies have reported that the serum ferritin level is not predictive of cardiac iron loading.[13,18] It has been reported that there isn't a correlation between the serum ferritin level and cardiac T2*.[6,19] However, some large-scale population-based studies reported that there is a weak correlation between the serum ferritin level and cardiac T2*.[20-22] In the present study there was a negative correlation between the serum ferritin level and cardiac T2* MRI values. The present findings also show that a high serum ferritin level was significantly correlated with low cardiac T2* MRI (p = 0.009), which is consistent with earlier studies.[19,23] Moreover, our result showed that there was negative correlation between serum ferritin level and all HRR values. These results may be important to reflect the relationship between autonomic dysfunction and myocardial iron accumulation.

Echocardiography is a valuable tool for monitoring cardiac functions in clinical practice, but it is not sufficiently sensitive for the early detection of cardiac involvement in beta TM patients,[24] and survival is already negatively affected by the time cardiac dysfunction is detected.[19,25] Moreover, even when the left ventricular ejection fraction is normal, the risk of cardiac decompensation due to iron overload cannot be ruled out.[26] Recent advances in echocardiography, such as TDI, are both sensitive and specific for predicting the presence of myocardial iron load in beta TM patients.[27,28] Diastolic dysfunction was evaluated via TDI in the present study and a negative correlation was noted between cardiac T2* values and diastolic dysfunction. The present findings show that a low cardiac T2* MRI value was significantly correlated with diastolic dysfunction (p = 0.03). Diastolic dysfunction of the heart usually appears prior to systolic dysfunction. In the present study, 7 patients with pure cardiac diastolic dysfunction had a normal T2* MRI (T2* > 20 ms). Ozbek et al.[29] reported that diastolic function can be impaired due to cardiac siderosis even when T2* is > 20 ms; therefore, TDI echocardiography can be used to diagnose cardiac involvement in beta TM patients earlier than cardiac T2*.

Myocardial biopsy for determining patient iron level is an invasive method that is not associated with cardiac iron levels or cardiac functions,[30] which may be related to the non-homogenous distribution of myocardial iron deposition.[31] As such, myocardial biopsy is not recommended for evaluating cardiac iron overload.

Deterioration of autonomic functions in beta TM patients has been reported.[32] In a recent study autonomic function was evaluated via 6 quantitative automatic function tests, and the prevalence of subclinical autonomic function disorder was higher in beta TM patients than in controls,[32] which confirms that there is some level of autonomic dysfunction in patients with beta TM. HRR has not been evaluated for the early detection of cardiac involvement in beta TM patients. Findings of the present study showed that high HRR1, HRR2, and HRR3 values were significantly correlated with high cardiac T2* (p < 0.001). Based on these findings, we think that the absence of a correlation between HRR4 and HRR5 values, and T2* was related to recovery of the heart to its normal autonomic functions after exercise, and that deteriorated HRR during the first 3 min of exercise testing in patients with beta TM may be a predictor of early cardiac involvement. Furthermore, the lack of evidence of abnormal CMR T2* with reduced HRR indicates that a significantly reduced HRR could be an early indicator of the preclinical stage of heart disease in beta TM patients.

The present study had several limitations. This was a single-center study and the patient population was not sufficient for determining the HRR status in patients with high and low cardiac T2* scores. Parameters such as heart rate variability and gas analysis were not considered during exercise testing. Exercise capacity, METs, and exercise duration were measured in min in this

study, and symptom-limited exercise duration was used instead of maximal exercise duration.

CONCLUSIONS

In conclusion, although CMR T2* is now recognized as the method of choice for evaluating iron deposition in the heart,[33] HRR might be used as an alternative approach for assessing cardiac involvement in beta TM patients. As it is widely available and considerably less expensive than CMR T2*, exercise training for HRR analysis can be performed in most providing hospitals. However, more data are needed to validate its use before it can be applied in clinical practice. Further studies are also needed to delineate the correlation between HRR and CMR T2*, as well as the clinical application of HRR as a predictive marker for autonomic dysfunction in beta TM patients.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict or financial interest associated with the publication of this article.

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