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Myocardial iron deposition in β-Thalassemia studied by magnetic resonance imaging

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Abstract

Myocardial iron deposition is a common finding in β-thalassemia. The iron content of the myocardium was assessed using the T2 relaxation time of the heart. The T2 relaxation time of the liver and skeletal muscle was also assessed in order to study the relation of iron deposition between heart, liver and skeletal muscle. ECG gated spin echo images were obtained from thirty-eight consecutive adult thalassemic patients examined in an outpatient clinic, aged (x±SD) 25±6 years, using a 0.5 T system. Patients were divided into groups A and B, according to their average serum ferritin levels of the preceding five years (> or < 2000 ng/ml). Results were compared with nine controls, aged 24±7 years. Heart T2 relaxation time in the control group (x±SD)(48.3±5.5 msec) was higher compared to group A (28.4±6.7 msec, $p<0.001$) but not to group B (43.4±7.4 msec). The T2 relaxation time of the heart correlated positively with the T2 relaxation time of the liver ($r=0.68$, $p<0.001$) and negatively with ferritin levels ($r=-0.67$, $p<0.001$). There was no correlation with the T2 relaxation time of skeletal muscle. This study indicates that regularly transfused β-thalassemia patients may present with a broad variation of heart iron deposition which, however, is related to serum ferritin levels.

Introduction

The thalassemias are a diverse group of congenital disorders in which there is a defect in the synthesis of one or more of the subunits of hemoglobin. In β-thalassemia, the β-chains of hemoglobin have a normal structure but are produced in reduced or, occasionally, undetectable amounts. The gene frequency for β-thalassemia approaches 0.1 in Southern Mediterranean areas and both sexes are equally affected. Diagnosis is based on detection of an abnormal hemoglobin pattern by electrophoresis. Patients present all signs and symptoms of severe anemia, including findings related to intramedullary and peripheral hemolysis, and iron overload. Adequate blood transfusions and continuous chelation are the milestones of treatment. However, the combination of chronic hypoxia and myocardial siderosis leads to cardiac arrythmias, congestive heart failure and, finally, death. Currently, bone marrow transplantation is performed in eligible cases, and gene therapy offers a promise for the future. Iron deposition in the heart and other organs is the causative factor of the main complications in β-Thalassemia Major [1–4, 27]. Excessive iron is primarily retained in the reticuloendothelial system and, when the capacity of this system is exceeded, secondary deposition in parenchymal organs will follow [5].

The estimation of iron stores in each individual organ and the total iron load in β-thalassemic patients would be helpful in order to evaluate the efficacy of chelation therapy. However, this is difficult to be assessed since hemosiderin and ferritin, the iron storage proteins, are mostly intracellular. On the other hand, serum ferritin is highly correlated with the amount of iron deposited in the tissues and is considered to be a very sensitive index [6]. Ferritin levels, however, may be affected by factors such as fever or inflammation [7, 8]. Presently, the most accurate way to estimate total body iron deposition is the direct measurement of iron content in liver biopsy specimens [9],

Table 1. Study group stratification (values as x±SD or x(range)).

	Group A	Group B	Group C
Subjects (n)	21	17	9
Ferritin (ng/ml)	4357 (2500–9000)	1550 (1060–2000)*	–
Blood units			
Transfused (n)	1025 ± 274	965 ± 429	–
Chelation (yrs)	13.3 ± 4.4	12.7 ± 5.0	–

* $p<0.01$.
Group A: high ferritin, Group B: low ferritin, Group C: controls.

an invasive procedure which can not be applied for routine follow-up and does not give any information about iron deposition in the heart. The ability of stored intracellular tissue iron to enhance magnetic susceptibility is the basis by which it can be detected by magnetic resonance imaging (MRI). Recent studies in experimental animals have shown that the T2 relaxation time has a linear correlation with the total iron contents for all organs, including the heart [10].

The aim of this study was to assess heart iron content using the T2 relaxation time of the heart, and to compare it to the T2 relaxation time of liver and skeletal muscle in a group of regularly transfused patients with β-thalassemia major, and also to correlate these parameters with the average serum ferritin levels of the last five years.

Patients and methods

Patient population (Tables 1, 2)

Thirty-eight consecutive patients with β-thalassemia major, 16 males and 22 females, aged (x±SD) 25±6 years were studied. Thirty-one of them presented a normal heart function and seven were diagnosed with heart failure. All patients were transfused monthly to maintain haemoglobin levels at 10–13 g/dL. Transfusion therapy had started before the age of 5 years and iron chelation therapy with deferoxamine (IM, IV or SC administration) had started before the age of 15 years. A serum ferritin level below 2000 ng/mL was considered the target value for a patient successfully treated with deferoxamine [28]. The average serum ferritin level of each patient was derived from 30 values obtained bi-monthly over the preceding 5 years. Thirty of the patients were splenectomized. Patients were stratified into two groups, according to their average ferritin values (> or < 2000 ng/ml): Group A (n=21), with ferritin levels of (mean (range)) 4357 (2500–9000) ng/ml and group B (n=17), with ferritin levels of 1550 (1060–2000) ng/ml. The total number of transfusions and years of chelation therapy were similar in all groups. The patients were compared with 9 normal controls (Group C), aged 24±7 years. An informed consent was obtained from all subjects and

Table 2. Individual patient Ferritin levels (ng/ml).

Group	No	Fer. aver	Fer. min	Fer. max
	*1	9000	5000	9500
	2	7000	3000	10000
	3	7000	6000	7500
	*4	6500	5000	10000
	5	5500	4000	7000
	6	5000	4500	10000
	7	4500	4000	8000
Group A	8	4500	2000	5000
(high Fer.)	9	4000	3300	5600
[No: 1–21]	10	4000	2000	12000
	11	4000	3600	6700
	*12	3600	2600	7500
	13	3500	1250	6000
	14	3500	2500	5500
	*15	3000	3200	7000
	16	3000	800	3300
	17	3000	3000	8500
	18	2800	680	7000
	19	2800	1800	7500
	*20	2800	2300	6000
	21	2500	2000	4000
	22	2000	1500	6000
	23	2000	1100	5000
	24	2000	750	5000
	25	2000	1800	5000
	26	1800	480	2200
Group B	*27	1700	1100	2100
(low Fer.)	28	1500	1100	2700
[No: 22-38]	29	1500	940	3400
	30	1500	1062	4000
	31	1500	270	5000
	32	1500	1000	4000
	33	1400	650	2500
	34	1200	680	3500
	35	1200	600	2200
	36	1200	600	2500
	37	1100	1000	2500
	*38	1060	950	8700

aver: average, min: minimum, max: maximum.
* patients with symptomatic heart failure.

the study was approved by the local Ethics Committee of the hospital.

MR techniques

Imaging technique

All MR studies were performed using a 0.5 T superconducting imaging system (MR-Max-Plus, GE/CGR). Body quadrature coil was used for both excitation and signal detection. The single oblique orientation of the imaging slices (head-left to feet-right) was determined from a scout coronal T1 localiser image in order to depict a short axis view of the heart. These slices were chosen since the relative images that they represented depicted the heart, liver and skeletal muscle (*latissimus dorsi*) in one picture. The slice with the best left ventricular delineation was selected. The MR study was ECG-gated with TR=heart rate, slice thickness 10mm and TE=12-120 msec in ten symmetrically repeatable echoes. These parameters were chosen on the basis of phantom studies, where a mean accuracy of 5.5% and a mean reproducibility of 4.5% was demonstrated for T2 values ranging from 10 up to 80 ms. For both sequences, the field of view was 42 cm and the image reconstruction matrix was 160×224. The longer anatomical axis was chosen as the frequency encoding axis, and the shorter as the phase encoding axis. Respiratory phase encoding and presaturation pulses were used to compensate for respiratory motion and blood flow artifacts respectively. Rectilinear pixels were used in order to improve the signal to noise ratio.

Quantitative image analysis

T2 relaxation time measurements for the left ventricle of the heart (T2LV), the liver (T2LI) and the skeletal muscle (T2MU) were performed from the T2 calculated imaging maps. These imaging maps were calculated using 10 successive T2-weighted images, obtained using the afore-mentioned single-slice multi-echo imaging technique. The T2-weighted MRI images were transferred from the MRI machine to a PC-workstation using appropriately selected hardware (calibrated HI-RES video frame grabber). Images were then archived as conventional PC-based imaging files (256 grey level, TIFF format, LZW compression).

Assuming single exponential behaviour of all tissues presented on conventional spin-echo images, a three point fit (in-house created fitted algorithm) was used. T2 calculated imaging maps were finally obtained on a pixel-by-pixel basis. The method applied for the needs of this study is the imaging representation of the quantitative T2 analysis (T2-QMRI) technique, applied partially by certain of the authors elsewhere [11]. T2 values were calculated from the imaging maps, using five appropriately selected regions of interest (ROIs), with each one situated on the chosen organ (heart, liver, muscle) for every patient. Observers were unaware of the serum ferritin measurements. The intra-observer and inter-observer coefficient of variation for T2 measurements from the imaging maps was 7% and 13% respectively.

Table 3. T2 relaxation time in the study groups (values as x±SD).

Groups subjects	Group A (n=21)	Group B (n=17)	Group C (n=9)
T2 heart (msec)	28.4±6.7	43.4±7.4[a]	48.3±5.5[a]
T2 liver (msec)	22.3±6.7	27.1±6.6[b]	34.9±5.0[c]
T2 muscle (msec)	28.4±2.7	29.2±2.4	30.0±1.9

[a] $p<0.001$, compared to group A
[b] $p<0.05$, compared to group A
[c] $p<0.01$, compared to group A and B
Group A: high ferritin, Group B: low ferritin, Group C: controls.

Statistical analysis

The results are expressed as x±SD (or x (range)) and compared by means of an unpaired two-tailed Student's t-test. The chi-square test was used for comparisons of percentages between groups. Correlations between various parameters were sought with Pearson's correlation coefficient. Statistical significance was considered for $p<0.05$.

Results

The T2 relaxation time of the left ventricle (T2LV) in the high ferritin group (group A) was 28.4±6.7 msec, in the low ferritin group (group B) it was 43.4±7.4 msec and in the control group (group C) it was 48.3±3.5 msec. T2LV in group C was higher compared to group A ($p<0.001$) but not compared to group B. There also was a significant difference between groups A and B ($p<0.001$) (Table 3 and Figures 1, 2).

There was a significant difference in the T2 relaxation time of the liver (T2LI) between groups A and B (22.3±6.7 vs. 27.1±6.6 msec, $p<0.05$). T2 relaxation time in both populations was lower compared

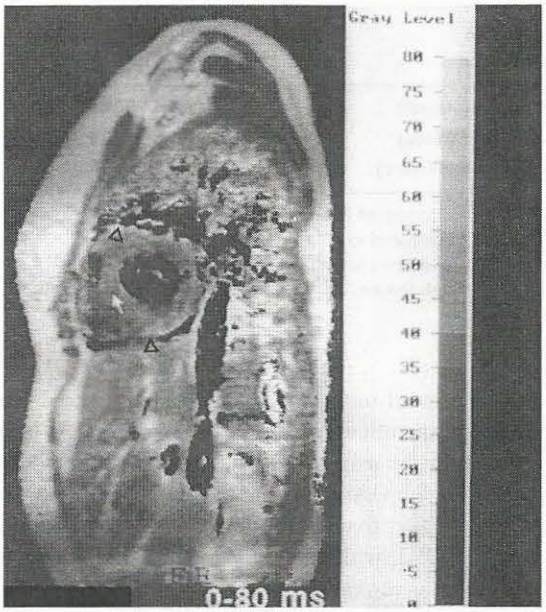

Figure 1. Short axis view of the heart of a thalassemic patient with mild iron overload. Iron deposition can be visualized as scanty black patches over the LV surface (left ventricle between arrowheads).

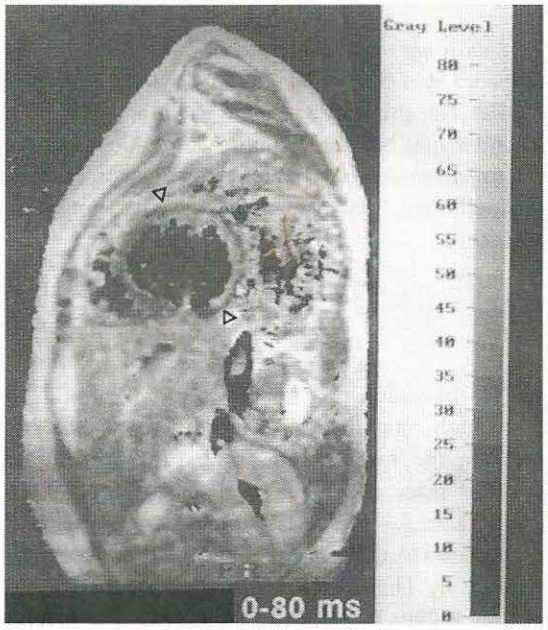

Figure 2. Short axis view of the heart of a thalassemic patient with severe iron overload and impaired LV function (dilated cardiomyopathy). The enlargement of the LV is accompanied by wide-spread black areas of iron deposition (left ventricle between arrowheads).

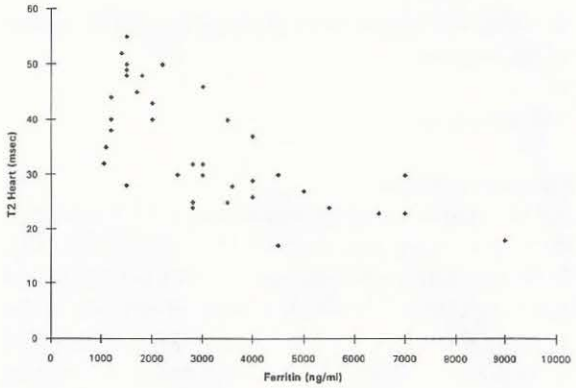

Figure 3. Inverse relationship between the T2 relaxation time of the heart and the average ferritin levels of the thalassemic patients ($r=-0.67$, $p<0.001$).

to controls in group C (34.3±5 msec, $p<0.01$). The T2 relaxation time of the skeletal muscle (T2MU) was similar in the three groups.

Five patients in group A (23.8%) exhibited heart failure, compared with only 2 in group B (11.8%, p=NS). There was no difference between patients with or without heart failure for T2LV (30.0±8.5 vs. 36.2±10.4 msec, p:NS), T2LI (21.4 ±5.9 vs. 25.1±6.5 msec, p:NS), T2MU (31.5±6.0 vs. 35.2±5.9 msec, p:NS) and ferritin levels (x (range)) (3951 (1060-9000) vs. 2909 (1100-7000) ng/ml, p:NS).

A positive correlation was found between heart and liver T2 relaxation times of the thalassemic population in our study ($r=0.68$, $p<0.001$).

The average ferritin levels of the preceding five years were inversely correlated with both the T2 relaxation time of the heart ($r=-0.67$, $p<0.001$) (Figure 3) and the liver ($r=-0.58$, $p<0.001$). No correlation was found between the T2 relaxation time of the skeletal muscle and ferritin levels.

Discussion

The presence of iron affects tissue T1 and T2 relaxation times. The effect on T1 relaxation time, however, is less significant and most studies use the T2 relaxation time to assess iron deposition [12]. This effect is proportional to the tissue iron content and depends on the applied magnetic field (B_o) [13]. We employed this principle to evaluate a group of adult thalassemic patients.

A 0.5 T imaging system enabled the implementation of a ten echo pulse sequence, in which an ear-

ly TE=12ms and several repeated TEs were applied. Errors were eliminated by measuring five regions of interest for each site. The signal to noise ratio was increased by a shorter TE, as suggested by other authors [12].

In our patient population, the heart and liver T2 relaxation times were significantly reduced, with higher iron concentrations resulting in lower T2 values. There was a significant difference in the heart T2 relaxation time between the high and low ferritin groups, but not between the controls and the low ferritin group. This indicated that the myocardium is usually unaffected in the early stages of iron deposition. Heart failure, a fatal complication of β-thalassemia, was present in both the high and low ferritin groups, possibly due to other factors than iron involvement in the pathogenesis of heart failure [14].

The liver T2 relaxation time was severely affected in both the high and low ferritin groups, compared to controls. This finding is suggestive of early and severe liver iron overload, even in cases with low total iron deposition.

Serum ferritin is currently the most accurate index of body iron content. Variations correspond to changes in the reticuloendothelial system storage iron but not to parenchymal iron content [15]. Liver disease, inflammation, infection and assay problems may influence measurements [7, 8, 16]. The average ferritin of the preceding five years was used in order to overcome these limitations. These levels inversely correlated with both heart and liver T2 relaxation times. The positive correlation between heart and liver T2 relaxation times suggests that both organs have a similar iron deposition pattern, although the liver seems to be more affected. The absence of changes in the skeletal muscle T2 relaxation time indicates that this muscle is unaffected by iron overload.

There is a controversy about the precise relationship between the myocardial iron content and the degree of heart dysfunction. Zaino et al. [17], using nuclear resonance scattering (NRS), found symptomatic cardiac disease in the patients with the highest cardiac levels of iron. Correlations between the heart T2 relaxation time and cardiac biopsy are absent since right ventricular biopsy is subject to serious sampling errors and iron deposition is patchy and not uniform [18]. A good correlation was found between iron content and T2 relaxation time only when whole rat hearts were sampled [10]. Recently, studies of thalassemic patients using 0.5 T indicated that cardiac complications are related to a low heart T2 relaxation time [19]. Additionally, the heart/muscle signal intensity ratio was sensitive to iron in patients treated by multiple blood transfusions [20] and impaired heart function was evident in the more heavily transfused patients, although there was no correlation with serum ferritin levels. MRI is useful in addressing this issue. In our study, heart failure in the low ferritin group suggests that iron deposition may not be the only factor for the development of heart failure [14].

Hepatic iron load, as studied by MRI, has been considered as an alternative to liver biopsy to assess the total iron load [21]. Quantitative image analysis (T2-QMRI) is a reliable technique, well correlated with liver biopsy [11]. *In vitro* measurements of the T2 relaxation time of liver samples from iron-overloaded rats [22] and spleen samples from thalassemic patients [12], demonstrated a linear correlation between relaxation rate (1/T2) and iron content. The reticuloendothelial system preferentially accumulates iron from the breakdown of transfused erythrocytes, before deposition occurs in parenchymal organs, e.g. heart. Our data are consistent with Buja et al. [23] who indicated that cardiac iron deposition is accompanied by heavy iron deposits in the liver.

Some authors consider the application of the T2 relaxation time in assessing heart iron deposition to be imprecise since it may be unmeasureable in severly iron-overloaded patients, due to low signal intensity equal to background noise [20]. The use of a 0.5 T machine (less prominent magnetic susceptibility phenomenon) [13], the use of shorter echo times (TE) [12] and the application of MR spectroscopy [21] appear to be more appropriate for the study of severe iron overload. Other non-invasive techniques such as dual energy computed tomography [24, 25], superconducted quantum interference device (SQUID) [26] and nuclear resonance scattering (NRS) [17] can overcome this limitation.

In conclusion, this study shows that T2-QMRI is a non-invasive tissue characterization method enabling the simultaneous examination of heart function, and heart and liver iron content. It is easily repeated, facilitating thalassemic patient follow-up and chelation therapy efficacy evaluation.

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