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T2 relaxation time study of iron overload in b-thalassemia

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Abstract

Myocardial iron deposition occurs as a result of blood transfusion therapy in b-thalassemia major patients. Since this deposition causes various cardiac complications, it is of interest to assess the iron content of the myocardium in relation to the clinical picture of the patients. Two different MRI indices were used to achieve this purpose: the T2 relaxation time and the heart/skeletal muscle signal intensity ratio. ECG gated spin echo images were obtained from 54 adult thalassemic patients, with a mean age of 26 (18–44) years, at TE = 22 ms and 60 ms, using a 1.5 T system. Patients were divided into 2 groups (A and B), according to their serum ferritin levels ($>$ or $<$ 2000 ng ml^{-1}). Results were compared with nine controls, with a mean age of 25 (18–43) years. Heart T2 relaxation time in controls (44.3 $\pm$ 3.5 ms) was higher than in group A (29.9 $\pm$ 5.7 ms, $P < 0.001$) and group B (33.4 $\pm$ 6.8 ms, $P < 0.01$). T2 was measurable in 66% of group A and 83% of group B patients. The heart/muscle signal intensity ratio in group A (0.45 $\pm$ 0.27) was lower than in group B (0.82 $\pm$ 0.33, $P < 0.001$) and the controls (1.15 $\pm$ 0.20, $P < 0.001$). The heart/muscle signal intensity ratio was measurable in 94% of the patients and demonstrated an inverse relationship with the serum ferritin levels ($r = -0.52$, $P < 0.01$). This study indicates that the heart/muscle ratio is a sensitive index of iron overload and it can be measured in the majority of patients, irrespective of tissue iron concentration, thereby offering an advantage over the use of T2 relaxation time.

Keywords: b-Thalassemia; Heart; T2 relaxation time

1. Introduction

b-Thalassemia major is a genetic hemoglobinopathy which is especially prevalent in Mediterranean countries. It is characterised by various degrees of ineffective hemopoiesis and intramedullary hemolysis. Current therapy includes regular blood transfusions with simultaneous iron chelation therapy, mainly employing desferrioxamine, in order to avoid secondary hemosiderosis. Bone marrow transplantation offers an effective alternative to constant blood transfusions, where the circumstances are appropriate for it to be performed. Gene therapy offers a bright outlook to the future treatment and annulment of the disease and its complications. Despite the benefits of iron-chelation therapy, iron deposition occurs in various organs, as a result of inadvertent iron overloading from the transfused blood. Iron deposition in the heart and other organs is the causative factor of the main complications in b-thalassemia major [1–5]. Excessive iron is primarily retained in the reticuloendothelial system and, when the capacity of this system is exceeded, secondary deposition in parenchymal organs follows [6].

The estimation of iron stores in each individual organ and the total iron burden in b-thalassemic patients

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is useful in evaluating the efficacy of chelation therapy. This is a difficult task since hemosiderin and ferritin, the iron storage proteins, are mainly intracellular. On the other hand, serum ferritin levels are highly correlated with the amount of iron deposited in the tissues, and are hence considered a very sensitive index [7]. However, the levels may be affected by factors such as fever, inflammation, etc. [8,9]. At present, the most accurate way to estimate the total body iron deposition is the direct measurement of iron content in liver biopsy specimens [10], an invasive procedure which cannot be applied during routine follow-up, and which does not offer any information concerning iron deposition in the heart.

The ability of stored intracellular tissue iron to enhance magnetic susceptibility provides 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 is linearly correlated with the total iron content for all organs, including the heart [11]. However, T2 relaxation time calculation may prove to be impossible in severely iron-overloaded patients, due to a very low signal intensity which is similar to background noise [12].

The aim of this study was to assess heart, liver and skeletal muscle iron content in b-thalassemia major patients using two different indices (T2 relaxation time of the heart, liver and skeletal muscles, and the heart/muscle and liver/muscle MRI signal intensity (SI) ratios), and to correlate these parameters with the corresponding serum ferritin levels.

2. Patients and methods

2.1. Patient population (Table 1).

Fifty four thalassemic patients, 24 males and 30 females, with a mean age of 26 (18–44) years, were studied. Thirty nine of them had no symptoms of heart failure (NYHA I) whereas 15 were diagnosed with heart failure (NYHA II–III). All patients were iron-chelated regularly and 40 of them were splenectomized. A serum ferritin level below 2000 ng ml^{-1} was considered to be the target value for a patient successfully treated with deferoxamine [27]. Patients were stratified into two groups, according to their average serum ferritin values ($>$ or $<$ 2000 ng ml^{-1}) over the previous 5 years: Group A ($n = 30$) with mean ferritin levels of 4150 ± 1653 ng ml^{-1} and group B ($n = 24$) with mean ferritin levels of 1240 ± 366 ng ml^{-1}. The total number of transfusions and years of iron-chelation therapy were similar in both groups. The results from thalassemic patients were compared with those of nine normal volunteers (Group C; controls) with a mean age of 25 (18–43) years. An informed consent was obtained from all participants.

Table 1
Study group stratification (values as $x \pm$ SD or range)

	Group A	Group B	Group C
Subjects (n)	30	24	9
Age (yrs)	26 (18–36)	26 (18–44)	25 (18–43)
Ferritin (ng ml^{-1})	4102 ± 1541	$1150+381$[a]	—
Blood units transfused (n)	1025 ± 274	965 ± 429	—
Chelation (years)	13.3 ± 4.4	12.7 ± 5	—

Group A: high ferritin population.
Group B: low ferritin population.

2.2. MRI techniques

All MRI studies were conducted using a 1.5 T Siemens system (Magnetom, SP), with an ECG gated spin echo sequence (acquisition matrix = 256×256, FOV = 40×40 cm^2, number of slices, eight, TR equal to RR interval, and TE = 22 and 60 ms). The oblique orientation of the imaging slices was determined from scout coronal anatomical images (parameters as above with TE = 22 ms), in order to depict a short axis view of the heart. These images included the liver and a section of skeletal muscle (latissimus dorsi). The signal intensities of the heart (SI_H), the liver (SI_L) and the skeletal muscle (SI_M) were determined using circular regions of interest (ROIs). Two indices were employed: (a) The T2 relaxation time of the heart ($T2_H$), liver ($T2_L$,) and skeletal muscle ($T2_M$), calculated from the images collected at the two TE values [13]; (b) The heart/muscle (SI_H/SI_M) and liver/muscle (SI_L/SI_M) signal intensity ratios at TE = 22 ms. The skeletal muscle was used as an internal standard to form this ratio since iron deposition is minimal in skeletal muscle [12].

2.3. Statistical analysis

The results are expressed as $x \pm$ SD and were compared by means of the unpaired two-tailed Student 1 test. The Chi square test was used to compare percentages between groups. Correlations between various parameters were sought by employing Pearson's correlation coefficient. Statistical significance was considered for $P < 0.05$.

3. Results

The T2 relaxation time of the left ventricle of the

Table 2
T2 relaxation time in the study groups (values expressed as $x \pm SD$)

	Group A	Group B	Group C
Subjects (n)	30	24	9
T2 heart (ms)	29.9 ± 5.7 (20)	33.4 ± 6.8 (20)	44.3 ± 3.5 (9)[a,b]
T2 liver (ms)	24.3 ± 1.8 (3)	29.0 ± 10.3 (12)	30.0 ± 7.7 (9)
T2 muscle (ms)	28.4 ± 2.7 (30)	29.2 ± 2.4 (24)	30.0 ± 0.9 (9)

The numbers in the brackets denote the number of subjects in whom the T2 relaxation time was measurable.
Group A: high ferritin population.
Group B: low ferritin population.
Group C: controls.
[a] $P < 0.001$, compared to group A.
[b] $P < 0.01$, compared to group B.

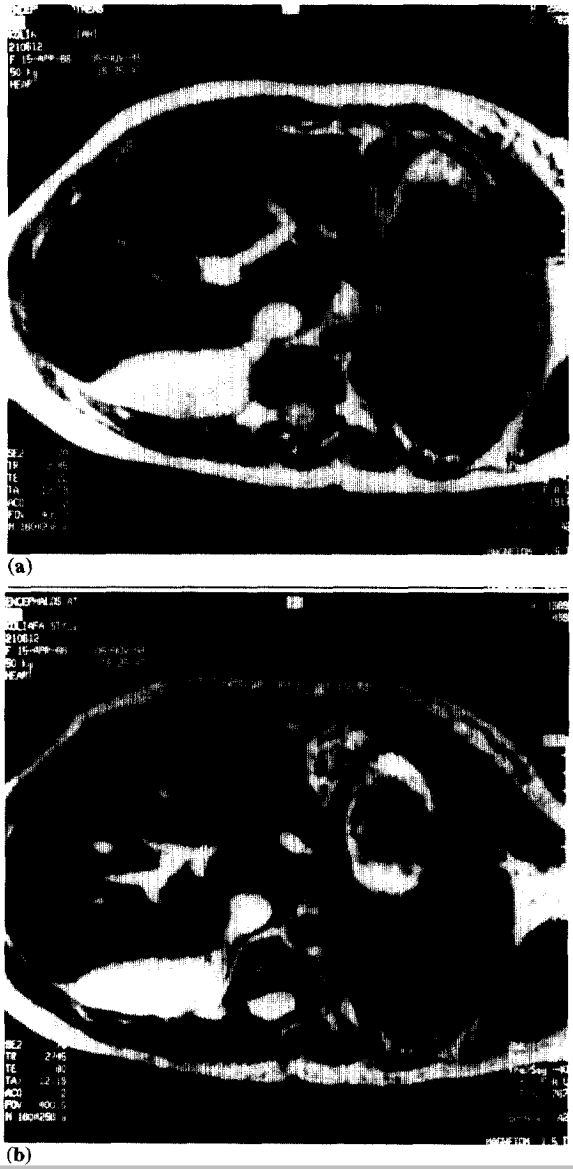
T2 relaxation time was $33.45 + 6.8$ ms and in the controls (group C), the time was 44.3 ± 3.5 ms. The $T2_H$ in group C was higher compared both to group A ($P < 0.001$) and group B ($P < 0.01$). There was no statistically significant difference between groups A and B (Table 2).

The signal intensity of the myocardium (SI_H) was only measurable at both echo times in 20/30 (66.7%) of the group A patients and in 20/24 (83.3%) of the group B patients (P:NS) (Fig. 1(a, b)Fig. 2(a, b)). As a result the $T2_H$ could only be calculated in these patients. Seven additional group A patients and the remaining four group B patients had measurable signal intensities at TE = 22 ms but not at TE = 60 ms. The signal to noise ratio was too low at both echo times to permit T2 and signal intensity ratio calculation in the final remaining three group A patients. Twelve group A patients (40%) and three group B patients (12.5%) manifested heart failure (NYHA II–III). Out of these patients, T2H was measurable only in four out of 12 of the group A population and in one out of three patients belonging to group B.

There was no difference in the T2 relaxation time of the liver ($T2_L$) between groups A, B and C. The signal intensities of the liver (SI_L) at both echo times and, consequently, the calculated $T2_L$, were only measurable in three out of 30 (10%) group A patients and 12 out of 24 (50%) group B patients ($P < 0.01$, chi square test). Twelve additional patients of group A and nine group B subjects had measurable signal intensities of the liver at TE = 22 ms, but not at TE = 60 ms.

The T2 relaxation time of the skeletal muscle ($T2_M$) was measurable in all subjects and was similar in the three groups. In group A, no correlation was documented between $T2_H$, $T2_L$ or $T2_M$ and ferritin levels, nor between the number of transfusions or iron-chelation time. In group B, an inverse correlation was found

The heart/muscle signal intensity ratio (SI_H/SI_M) in group A (0.45 ± 0.27) was lower than in group B (0.82 ± 0.33, $P < 0.001$) and group C (1.15 ± 0.2, $P < 0.001$) (Table 3). Similarly, the liver/muscle signal intensity ratio (SI_L/SI_M) in group A (0.17 ± 0.13) was lower than in group B (0.37 ± 0.31, $P < 0.01$) and group C (1.28 ± 0.16, $P < 0.01$). Patients with heart failure, as a subgroup, had a lower SI_H/SI_M ratio (0.33 ± 0.19) compared to the mean group A ($P < 0.001$) and group B ($P < 0.001$) values. Ferritin levels were inversely correlated with both SI_H/SI_M ($r = -0.52$, $P < 0.01$) (Fig. 3) and SI_L/SI_M ratios ($r = -0.41$, $P < 0.01$). In group B, a positive correlation ($r = 0.55$, $P < 0.01$) was found between the SI_H/SI_M ratio and $T2_H$ values.


(a)

(b)

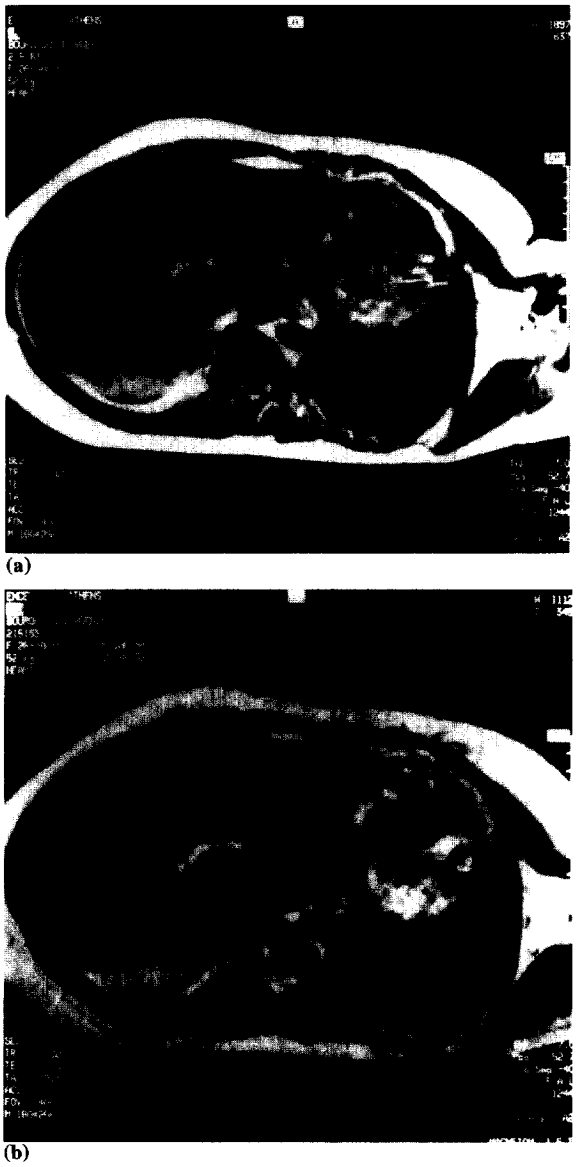


Fig. 2. (a and b) Short axis view of the heart of a thalassemic patient with severe iron overload and impaired LV function, using TE = 22 ms (a) and TE = 60 ms (b). In the latter image, the myocardial signal intensity is equal to the background noise.

4. Discussion

The presence of iron affects the tissue T1 and T2 relaxation times [14,15]. However, the effect on T1 relaxation time is not as significant [15], and most studies use the T2 time for characterization of iron deposition. This effect is proportional to the concentration of iron in the tissue and depends on the applied magnetic field (Bo) [16]. This concept was employed to evaluate a group of adult thalassemic patients.

In this study, the heart and liver T2 relaxation times were significantly reduced, with higher iron concentration. significant difference was found in the heart T2 relaxation time between the high and low serum ferritin groups. Serum ferritin levels were only inversely correlated to heart T2 relaxation times ($T2_H$) in the low ferritin group. These observations result from the fact that the T2 of several patients in the high ferritin group could not be measured due to low signal to noise ratio. On the contrary, signal intensity measurements were feasible in all patients, irrespective of iron burden. Significant differences were recorded between the two patient groups, and an inverse correlation was found between heart/muscle and liver/muscle SI ratio and serum ferritin levels in the thalassemic population. Since there is a significant error involved in the calculation of the T2 relaxation time when the tissue signal intensity (SI) is low, heart SI (normalized in proportion to skeletal muscle employed as the control standard) is a more appropriate index for correlation with iron deposits.

Heart failure, a serious complication of b-thalassemia, was present in both ferritin groups, although it was more prevalent in the severely iron-overloaded group. When heart failure patients from groups A and B were analysed as a separate sub-group, the T2 relaxation time of the heart was measurable only in one third of the population, and the heart/muscle signal intensity ratio was even lower than the values observed in the high ferritin group. Similarly, the T2 relaxation time of the liver was measurable in half of the patients in the low ferritin group, whereas in the majority of the patients in the high ferritin group, measurements proved to be impossible. When the liver/muscle signal intensity ratio was employed, however, measurements in all patients were achieved and differences were manifested between the high and low ferritin groups, and the control subjects.

Table 3
Signal intensity ratios (SI) in the study groups (values expressed as $x \pm SD$)

	Group A	Group B	Group C
Subjects (n)	30	24	9
SI_H/SI_M	0.45 ± 0.27 (27)	0.82 ± 0.33 (24)[a]	1.15 ± 0.20 (9)[b]
SI_L/SI_M	0.17 ± 0.13 (15)	0.37 ± 0.31 (21)[c]	1.28 ± 0.16 (9)[c]

Signal intensities measured at TE = 22 ms (H: heart, M: skeletal muscle, L: liver). The numbers in the brackets denote the number of subjects in whom the signal intensity ratios were measurable.
Group A: high ferritin population.
Group B: low ferritin population.
Group C: controls.
[a] $P < 0.001$

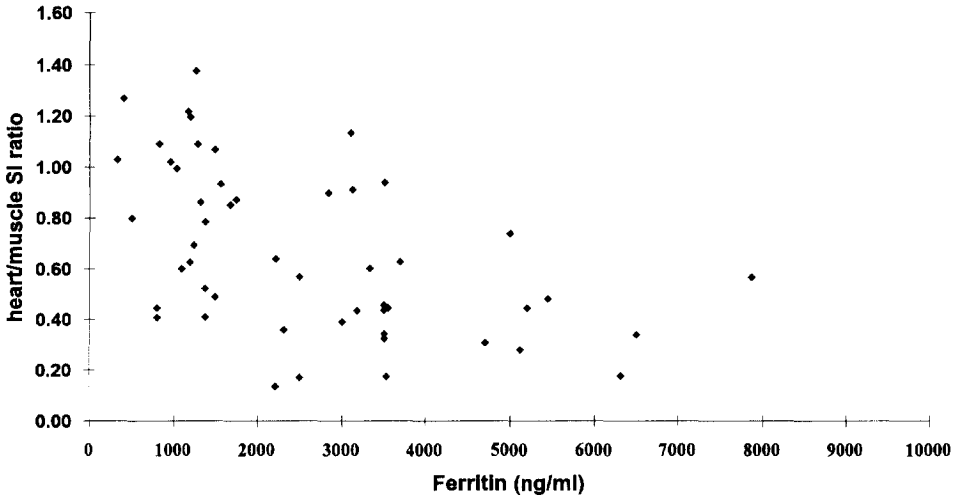


Fig. 3. Inverse relationship between the heart/muscle signal intensity (SI) ratios and the average ferritin levels of the thalassemic patients ($r = -0.51$; $P < 0.01$).

Serum ferritin is currrently considered the most accurate index of body iron content. Variations in serum ferritin mainly correspond to changes in the reticuloendothelial system storage iron levels and not to changes in the parenchymal iron content [17]. Liver disease, inflammation, infection and assay complications [8,9,18] have been reported to influence measurements. In this study, in an attempt to overcome these limitations, the average ferritin levels of the preceding five years were employed.

There is a controversy over the precise relationship between the quantity of iron present in the myocardium and the degree of heart dysfunction. Correlations between heart T2 relaxation time and cardiac biopsy are absent since right ventricular biopsies are subject to serious sampling errors and iron deposition is patchy (not uniform) [19]. A good correlation was found between iron content and T2 relaxation time only when whole rat hearts were sampled [11]. Under these circumstances, MRI appears to be useful in addressing this issue.

Recently, studies of thalassemic patients at 0.5 T indicated that cardiac complications are related to low T2 times [20]. The heart/muscle signal intensity ratio was also found to be sensitive to iron content in a group of patients treated by multiple blood transfusions [12], and a trend for worse heart function was evident in the more heavily-transfused patients, even though there was no correlation with serum ferritin levels. In addition, Zaino et al. [21], employing a new in vivo, non-invasive method for measuring iron utilizing nuclear resonance scattering (NRS), detected symptomatic cardiac disease in the patients with the more elevated cardiac iron levels.

of total iron burden [22]. In vitro measurements of T2 relaxation time of liver samples, from iron-overloaded rats [14] and spleen samples from thalassemic patients [15], demonstrated a linear correlation between relaxation rate (1/T2) and iron content. The reticuloendothelial system preferentially accumulates iron from the breakdown of transfused red blood cells before iron deposition occurs in other parenchymal organs such as the heart. The data presented here are consistent with the study by Buja et al. [23], which indicates that cardiac iron deposition is accompanied by excessive hepatic iron deposits.

Although the T2 relaxation time is generally a reliable index of iron deposition, being well-correlated with liver biopsy information, it may be unmeasurable in severely iron-overloaded patients, due to low signal intensity equal to background noise. For this reason, some authors consider the application of the T2 relaxation time to assess heart iron deposition as being inaccurate [12]. The use of a 0.5 T machine, where the magnetic susceptibility phenomenon is less prominent [16], the use of shorter echo times (TE) [15] or the application of MR spectroscopy [22], could be more promising in the study of severe iron-overloaded patients. Other non-invasive techniques such as dual energy computed tomography [24,25], superconducted quantum interference device (SQUID) application [26], and nuclear resonance scattering (NRS) can overcome this limitation, but are not available on a wide scale.

This study suggests that MRI could prove to be an effective non-invasive method in performing tissue characterization. MRI, in particular, provides the means for the simultaneous examination of heart iron deposition and heart function. Moreover, measurements are quantifiable, enabling the repeated examina-

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