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IRON OVERLOAD IN THALASSEMIA: COMPARATIVE ANALYSIS OF MAGNETIC RESONANCE IMAGING, SERUM FERRITIN AND IRON CONTENT OF THE LIVER

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ABSTRACT

Background. **Iron overload in patients with thalassemia is a common feature which requires continuous chelation therapy and monitoring. Serum ferritin determination is widely accepted as a simple method for following iron load in patients with primary hemochromatosis; however, several reports on thalassemic patients emphasize that ferritinemia is not accurate and that other methods such as direct measurement of iron in the liver (HIC) and magnetic resonance imaging (MRI) are more precise.**

Materials and Methods. **In order to contribute to the general understanding of iron load in thalassemia we used liver MRI to study 33 thalassemic patients, most of whom were also evaluated for iron content by liver biopsy. The data were then compared with serum ferritin levels.**

Results. **Ferritin levels ranged between 276 and 8031 ng/mL, and liver iron content ranged from 1.6 to 31.0 mg/g dry weight; grade III and IV liver siderosis was recorded in 23/33 patients, just as 23/33 patients were found to have severe or very severe siderosis at MRI. Significant correlations with ferritin levels were recorded between grade IV and grades III, II and I ($p<0.01$, $p=0.02$, and $p=0.03$, respectively). Ferritinemia also showed significant linearity with liver iron content ($r=0.603$, $p=0.001$). No significant differences of ferritin levels were recorded, however, between patients found to have severe and those with mild iron load at MRI ($p=0.073$).**

Conclusions. **Our study shows that serum ferritin levels exhibit a tendency to be significantly correlated with the true status of hemochromatosis in thalassemic patients; however, the discrepancies recorded in several patients and the scarce or total lack of correlation with MRI led us to reconsider a future approach to this problem in order to arrive at a correct decision on therapy.**

Key words: thalassemia, liver, ferritin, MRI, iron overload

Hepatic iron concentration (HIC) is the most useful method for estimating iron load in chronically transfused patients.[1-5] However, while the method is generally safe, it requires a liver biopsy and an undefinible risk of morbidity (and rarely of mortality) is reported.[6] Thus alternative procedures for detecting iron load in the body in order to make proper decisions concerning chelation therapy are warranted. Attempts to correlate serum ferritin with HIC have failed to demonstrate a linear relationship between the two parameters[7,8] and discrepancies have frequently been observed. Other studies have focused on evaluating whether magnetic susceptometry (SQUID) and magnetic resonance imaging (MRI) or other imaging techniques could be proposed as a substitute for HIC in monitoring iron in the liver and in other

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tissues, but convincing results were obtained only in cases where hepatic iron levels were five times greater than the normal upper limit.[8-17]

With the aim of contributing to the general understanding of iron overload in thalassemia, we report our experience on a group of thalassemic patients whose hepatic iron levels were studied by MRI, liver biopsy and HIC in most cases. Biopsy and HIC data were compared with serum ferritin; in addition, a correlation between qualitative MR images and serum ferritin was also attempted.

Patients and Methods

Thirty-three patients with homozygous β-thalassemia who underwent liver MRI formed the basis of this study. The onset of transfusion in these patients occurred between the ages of 3 months and 7 years, and all were under chelation therapy with DFO at the time of the study: 50 mg/kg/day, five to six days a week. Ages ranged between 5 and 29 years, mean 19.6 years; 22 patients were males and 11 females.

Ferritin values

Determination of serum ferritin levels was performed immediately prior to liver biopsy in all patients under study; the method used was a fluorimetric enzyme immunoassay (Baxter-Stratus).

Liver biopsies

Liver biopsies were performed with a 14-gauge Klatskin-modified Menghini needle (Becton-Dickinson, East Rutherford, NJ, USA) using a standard aspiration technique. Biopsies and other studies were carried out during the final three months of observation, with a maximum interval of 8 weeks between liver biopsy and MRI. Biopsies consisted of a tissue core at least 2 cm in length; the more superficial half of the specimen was sent to our pathology department for routine histological studies, while the other half went to the laboratory for chemical analysis (see below). Each specimen was stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-Schiff with diastase, reticulum, and prussian blue stain. All biopsy specimens were observed by two of the authors (DMS, SV) and graded for the presence and degree of fibrosis, inflammation, necrosis, fat and iron. The amount of stainable iron present was graded from 0-4 according to the method of Scheuer *et al.*[18]

Hepatic iron concentration (HIC): analytical procedure

The deeper portion of liver biopsy specimens was collected in polypropylene tubes previously tested for internal surface iron contamination; empty tubes were conditioned at 80°C for 24 hours, and then conditioned again at room temperature for 4 hours and weighed. Variations in tube weight were evaluated by inserting some empty tubes in each group of samples. Each sample was leached with 0.5 mL concentrated HNO3 at 60° C for 60' and diluted to 2.5 mL with distilled water. Solutions were analyzed with three different methods: spectrophotometry (ferene-pH 4.5), flame atomic absorption, and flameless atomic absorption. Iron levels obtained with the different methods showed very good correlation ($r > 0.99$), especially ferene and flame-AA results, while the flameless-AA method seemed to be more sensitive to iron contamination. Contamination during bioptic sampling was also evaluated by analyzing specimens of animal liver collected with the same technique; it proved to be negligible.

Magnetic resonance imaging (MRI) studies

Magnetic resonance imaging was performed with a Toshiba (Japan) superconducting magnet operating at 0.5 T. The thorax and abdomen were included in the study field by using an FOV (field of view) that varied between 30 and 40 cm depending upon the body size of the subject. The thickness of slices was 10 mm.

Three series of images were obtained: 1) T1-weighted transverse images with a TR (time of pulse repetitions) equal to 80% of the R-R interval as measured on ECG, 4 measures and a TE (time of echo) of 20 msec. Depending on the TR, 13 to 18 slices with a gap of 1 to 3 mm were obtained; 2) proton density and T2-weighted transverse images, with a TR of 2500 msec, 2 measures and a TE of 40 and 80 msec,

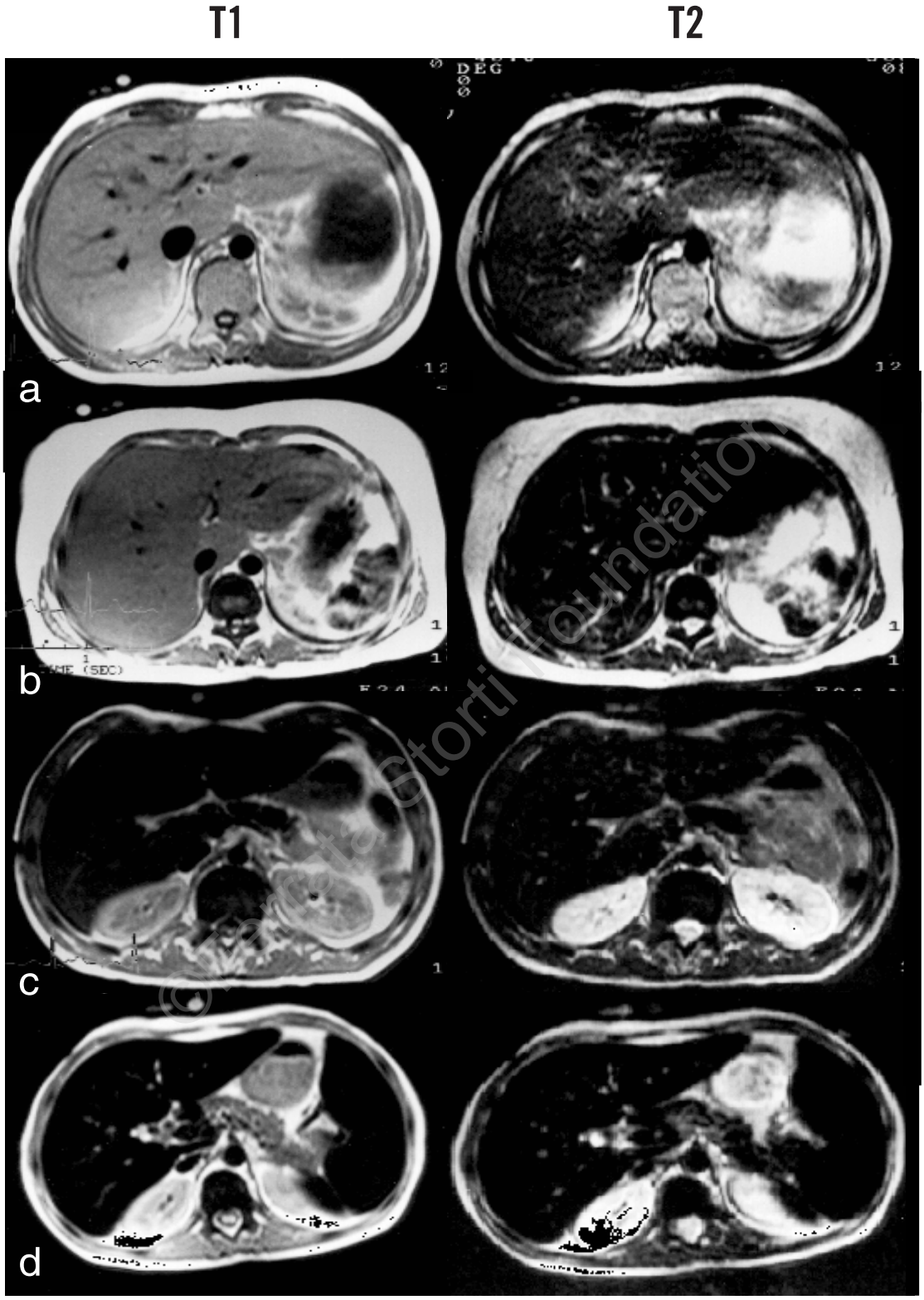

Figure 1. Images show the different grades of arbitrarily assigned siderosis. Left: T1 images of patients with mild, moderate, severe and very severe siderosis; right: T2 images of patients with mild, moderate, severe and very severe siderosis (a_{1-2}, b_{1-2}, c_{1-2}, d_{1-2}, respectively).

respectively; 17 slices with gap intervals of 1 to 3 mm were obtained; 3) T1-weighted coronal images with a TR equal to 80% of the R-R interval, 4 measures and a TE of 20 msec.

MRI signals were evaluated on liver, spleen, pancreas, bone marrow and myocardium. An arbitrary siderosis grading was established and is reported in Figure 1 (a,b,c,d): 0=absent (normal signal for T1- and T2-weighted images); 1=mild (normal signal for T1- weighted images and mildly reduced for T2-weighted images); 2=moderate (mildly reduced signal on T1-weighted and reduced signal for T2-weighted images); 3=severe (reduced signal for T1-weighted images and markedly reduced on T2-weighted images), and 4=very severe (markedly reduced signal for T1- and T2-weighted images).

Statistical analysis

Statistical analysis was carried out with the non-parametric tests that are considered the useful for abnormally distributed data due to a small patient sample: the Mann-Whitney U-test and Sperman's rank test. The former was employed for comparing the mean values of non dichotomous discrete variables, and the latter to evaluate the correlation index ($0 > r < 0.25$ = no correlation, $0.25 > r < 0.50$ = good correlation, $r > 0.75$ = optimal correlation).

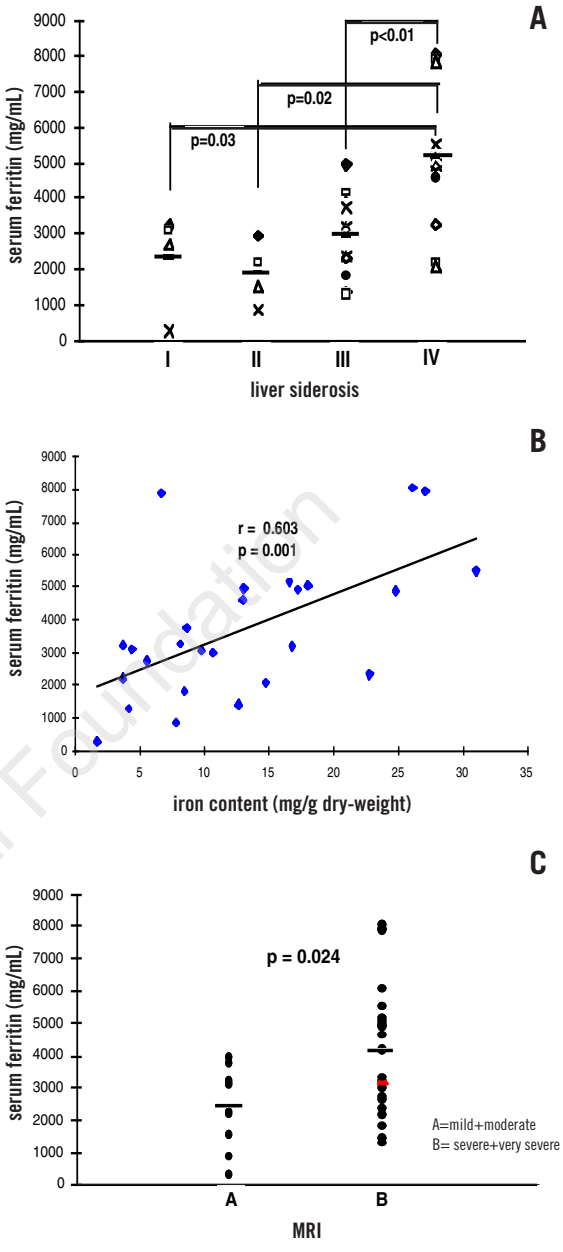

Figure 2. Comparative evaluation of different levels of serum ferritin with histological grade of siderosis (a), with HIC (b), and with MRI (c).

Results

Table 1 gives a detailed report of liver histology, iron content and MRI evaluation, as well as serum ferritin level, hepatitis, liver fibrosis and cirrhosis data for the 33 patients studied.

In summary, all patients underwent MRI, 31 had a liver biopsy and 26 had the iron content of the liver measured. Twenty-seven patients had been splenectomized. Ferritin levels ranged between 276 and 8031 ng/mL with a mean value of 3644 ng/mL. Liver siderosis as determined by standard histological examination showed 4 patients with grade I, 4 with grade II, 11 with grade III and 12 patients with grade IV; data were not available for two patients. HIC was measured in 26 patients; the mean value of wet liver samples was 2.52 mg (range 0.5-7.76 mg, SD=1.57). Patients whose wet tissue weight was less than 0.5 mg were excluded. HIC ranged between 1.6 to 31.0 mg/g dry tissue weight, with a mean value of 12.9 mg/g; 5 patients had a small core biopsy and were excluded from this evaluation. The grade of siderosis at MRI was judged to be mild in three patients, moderate in 7, severe in 18 and very severe in 5 patients.

Table 1. Variables examined in 33 patients with thalassemia.

No.	*Sex*	*Age (yrs)*	*Mean serum ferritin (ng/mL)*	*Liver biopsy: grade of liver siderosis*	*Liver iron content (mg/g)*	*Mri: grade of liver siderosis*	*Splenectomy*	*Notes*
1	M	17	8,031	IV	26.1	severe	yes	liver fibrosis
2	M	29	1,538	II	—	mild	yes	chronic hepatitis
3	M	13	5,044	IV	18	severe	yes	chronic hepatitis
4	F	17	5,500	IV	31	very severe	yes	liver fibrosis
5	M	20	276	I	1.6	mild	yes	cirrhosis
6	M	27	4,964	III	13	severe	yes	—
7	M	21	2,969	II	10.6	very severe	yes	chronic hepatitis
8	M	15	2,112	IV	14.7	severe	yes	liver fibrosis and hepatitis
9	M	15	3,770	III	8.6	moderate	yes	—
10	M	20	2,175	IV	—	moderate	yes	liver fibrosis
11	F	21	2,201	II	3.7	mild	yes	diabetes
12	F	29	3,223	I	3.7	moderate	yes	—
13	M	13	3,083	I	9.7	moderate	no	—
14	F	21	7,900	IV	27.1	severe	no	chronic hepatitis and fibrosis
15	M	23	859	II	7.8	moderate	yes	—
16	M	21	1,272	III	4.1	severe	yes	liver fibrosis
17	M	25	2,732	I	5.5	severe	yes	—
18	F	26	6,049	—	—	severe	yes	—
19	F	21	4,932	IV	17.2	severe	yes	chronic hepatitis
20	M	27	3,928	III	—	moderate	yes	chronic hepatitis
21	M	18	7,875	IV	6.6	severe	no	chronic hepatitis, liver fibrosis
22	F	16	4,139	III	—	severe	no	chronic hepatitis, liver fibrosis
23	M	19	3,200	III	16.8	very severe	yes	chronic hepatitis
24	M	5	2,609	—	—	severe	no	—
25	M	10	2,334	III	22.7	very severe	yes	—
26	M	18	5,162	IV	16.5	severe	yes	chronic hepatitis and cirrhosis
27	M	19	1,796	III	8.4	severe	yes	chronic hepatitis
28	F	25	3,119	III	4.3	moderate	yes	chronic hepatitis
29	F	16	4,591	IV	12.9	severe	yes	liver fibrosis
30	F	20	3,265	III	8.1	severe	yes	chronic hepatitis
31	M	21	1,396	III	12.6	severe	yes	liver fibrosis
32	F	20	4,900	IV	24.8	very severe	no	chronic hepatitis
33	M	18	3,300	IV	—	severe	yes	liver fibrosis

As shown in Table 1, liver fibrosis was detected in 11 patients, chronic hepatitis in 12 patients and cirrhosis in 2. Diabetes was reported in one patient.

Figure 2 (a, b and c) shows a comparative analysis of serum ferritin with histologically detected siderosis (a), with HIC (b), and with grade of siderosis at MRI (c). An analysis of data concerning the grade of siderosis showed a significant difference (Mann-Whitney U-test) between grade IV ferritin levels and those of grades III, II, and I ($p < 0.01$, $p = 0.02$, and $p = 0.03$, respectively), while no significant differences were recorded between grades III and II, grades III and I or between grades II and I.

The rank test was applied to HIC data and ferritinemia: $r = 0.603$ and $p = 0.001$, with a modest correlation between the two variables.

Analysis (Mann-Whitney U-test) of serum ferritin levels and MRI data was performed by

grouping patients with mild to moderate siderosis (group A) and those with severe and very severe siderosis (group B). A statistically non significant difference was observed between the ferritin levels of the two groups (p= 0.073), whereas the severity of liver siderosis at MRI was significantly different between the two groups (p = 0.003).

Discussion

In this study we focused our attention on whether the serum ferritin level could be considered a realistic parameter for the detection of iron overload in a population of thalassemic patients, most of whom were over 10 years old.

First we showed that there is a certain correlation between ferritinemia and liver iron content as measured directly in the tissue. Nevertheless, we saw serious discrepancies between the serum ferritin level and HIC in several patients.

Our second observation was that ferritinemia correlates significantly with histologically detected grade IV siderosis, but apparently not with less extensive iron loads. In fact, our data reveal a great variability in ferritinemia levels among patients with stage II and III siderosis (Figure 2a).

A third observation was that MRI provides a practical grading of the hepatic iron load based on the severity of the picture, albeit arbitrarily assigned (Figure 1). However, no significant correlation was seen with ferritinemia levels when different severities of siderosis were compared, although a trend toward significance was evident when the ferritin values of patients with less severe iron load were compared with those of thalassemic with greater iron overload.

We emphasize that our study presents data similar to those already reported concerning both comparisons between serum ferritin levels and direct measurement of hepatic iron, as well as the relevance of MRI in qualitative detection of iron load in patients with hemochromatosis.[10] However, there are few data regarding thalassemia with a comparative analysis of several methods for measuring hepatic iron load.[19] Nevertheless, we must conclude that ferritinemia is not always correlated with the true iron load status in thalassemic patients. The probable explanation for this observation may be obvious but further studies, which are in progress, are needed to demonstrate this conclusion.

First, ferritin is a protein which reflects the inflammatory status of a subject and which also increases in patients with cancer;[20-21] moreover, it is well known that in thalassemic patients, especially older ones, HCV-related chronic hepatitis is frequently associated.[22] Second, the grade of iron load is determined by visual evaluation and a standardized definition is difficult because it depends on the thickness of the intracellular iron granules. On the other hand, the distribution of the granules and whether they involve both Kupffer's cells and hepatocytes can easily be seen. As hepatic fibrosis progresses and inflammatory cells decrease the liver gradually loses its capacity to synthesize and release ferritin, and in the final stage of cirrhosis the ferritin level is generally low, as one of our patients demonstrated and as has been reported.[23]

Furthermore, the experience recorded by the most important thalassemia transplant team shows that after successful transplantation the progressive reduction of the iron load proceeds from the centrolobular area to the portal spaces, and the ferritin level could remain elevated until the process has been completed, depending on the persistence of inflammatory cells and/or the presence of chronic GVHD.[24] Various complications may further influence serum ferritin level following bone marrow transplantation.[25]

In summary, there are two main points to be stressed by this study: 1) serum ferritin, although emblematic of the body iron status of many thalassemic patients, is a non reliable indicator of the iron load in a substantial number of patients, most of whom suffer from inflammatory liver disease; 2) MRI seems to be an alternative to ferritinemia and studies on a larger number of patients are in progress. Direct iron measurement would undoubtedly be the best way to assess the severity of secondary hemochromatosis, and a way to compare the values obtained by these two methods is needed.

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