Transfusional iron overload in patients with sickle cell anemia: comparison between magnetic resonance imaging and serum ferritin*

Sobrecarga de ferro transfusional em portadores de anemia falciforme: comparação entre ressonância magnética e ferritina sérica

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Abstract **Objective:** To identify predictive variables of iron overload in patients with sickle cell anemia, correlating biochemical and imaging markers. **Materials and Methods:** The authors developed a cross-sectional study involving 32 patients with sickle cell anemia who were evaluated for ferritin and iron serum levels and submitted to liver magnetic resonance imaging with one spin-echo and five gradient-echo sequences. The signal intensity was obtained at each sequence, corresponding to the arithmetical mean of the measurements on regions of interest in the liver and paraspinal muscles to obtain the liver/muscle signal intensity ratio (SIR). Based on such SIR, the liver iron concentration (LIC) was estimated by means of the following formula: $e^{[5.808 - (0.877 \times T2^*) - (1.518 \times IW)]}$, where T2* is the SIR on the sequence with echo time = 13 ms and IW is the SIR on the intermediate-weighted sequence. Patients were grouped according to their blood transfusion regimen (regular monthly versus sporadic transfusions). **Results:** The comparison between the transfusion groups was based on clinical-laboratory variables, with significant differences in SIR, LIC and serum ferritin levels: the group with regular transfusions demonstrated greater hepatic iron overload. **Conclusion:** Magnetic resonance imaging is an efficient tool for evaluating liver iron overload in patients with sickle cell anemia.
Keywords: Sickle cell anemia; Magnetic resonance imaging; Ferritin.

Resumo **Objetivo:** Identificar variáveis preditoras de sobrecarga de ferro em portadores de anemia falciforme e correlacionar indicadores bioquímicos e imaginológicos. **Materiais e Métodos:** Foi realizado estudo transversal envolvendo 32 portadores de anemia falciforme, que foram submetidos a dosagem sérica de ferro, ferritina e a ressonância magnética do fígado. Foram realizadas cinco sequências gradiente-eco e uma spin-eco. A intensidade de sinal foi obtida em cada sequência pelas médias das regiões de interesse no fígado e musculatura paravertebral para obter a razão da intensidade de sinal (RIS) fígado/músculo. A partir da RIS foi obtida a concentração hepática estimada de ferro (CHEF) pela fórmula: $e^{[5,808 - (0,877 \times T2^*) - (1,518 \times PI)]}$, onde T2* é a RIS na sequência com TE de 13 ms e PI é a RIS da sequência com ponderação intermediária. Os pacientes foram agrupados segundo o regime de transfusão de hemácias (regulares mensais *versus* esporádicas). **Resultados:** Os grupos transfusionais foram comparados pelas variáveis clínico-laboratoriais, sendo significativas as diferenças entre RIS, CHEF e ferritina sérica: o grupo que recebeu transfusões regulares apresentou sobrecarga de ferro hepático mais intensa. **Conclusão:** A ressonância magnética foi ferramenta eficiente para avaliação de sobrecarga hepática de ferro em portadores de anemia falciforme.
Unitermos: Anemia falciforme; Imagem por ressonância magnética; Ferritina.

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INTRODUCTION

In the setting of acute complications of sickle cell anemia (SCA), the decrease in hemoglobin levels may precipitate a cardio-pulmonary function decompensation, so erythrocytes transfusion become a therapeutic resource of great importance[1]. With the frequent long term utilization of such resource, some complications such as iron overload may occur[2], as there is no active excretory mechanism for this micronutrient. Main manifestations of advanced iron overload include dysfunction of or-

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gans such as the liver, heart and endocrine organs.

There are direct and indirect methods to estimate body iron levels[3]. Serum ferritin test is the most available method and is easily reproducible, in spite of not providing a good specificity[3]. Hepatic biopsy, in spite of being validated as a reference standard, is an invasive method and, therefore, involves risks inherent to the procedure[4]. For these reasons, the possibility of replacing such a procedure by a noninvasive imaging method, in this case magnetic resonance imaging (MRI), is discussed[5].

Several studies have described the utilization of MRI in the quantification of hepatic iron content, with variable results among them[5–9]. Iron overload causes a decrease in the signal intensity of the hepatic parenchyma and the measurement of such an overload is possible since there is a correlation between the magnitude of signal reduction and the degree of iron excess[8].

The present study is aimed at estimating the liver iron concentration by means of MRI in patients with SCA, and correlating iron overload biochemical and imaging markers in two different transfusion regimens.

MATERIALS AND METHODS

A cross-sectional study was developed with SCA patients regularly followed-up at a reference outpatient clinic in the state of Sergipe, Northeastern region of Brazil. In this region, there is a high prevalence of the hemoglobin S gene because of the ethnic composition of the population[10]. For the purposes of the present study, eligible patients were those with SCA confirmed by means of hemoglobin electrophoresis and had already undergone blood transfusion. Patients with chronic hepatic disease not related to SCA were excluded.

Thirty-two patients were sequentially selected and divided into two groups: the first group comprised patients undergoing sporadic blood transfusions due to complications (SG), and the second group, comprising patients undergoing regular blood transfusions (RG): 10 to 20 ml/kg administered monthly because their history of cerebrovascular accident.

All the patients underwent clinical examination, laboratory tests (serum ferritin, serum iron, aminotransferase test and blood count) and MRI. Upper abdomen MRI scans were performed in a 1.5T Magnetom Avanto (Siemens; Munich, Germany) apparatus, without the use of contrast medium or sedation.

The parameters utilized for the sequences acquisition were based on the protocol proposed by Alústiza et al.[8]. The technique comprised five breath-hold GRE sequences (20 sections each), and one TSE trigger sequence (with respiratory synchronization and 35 sections), whose details are described on Table 1.

All the images were analyzed by a single experienced and duly trained radiologist. The regions of interest (ROIs), measuring at least 1 cm^2, were positioned on three areas of the right hepatic lobe for measurement of the signal intensity and on two areas of the paravertebral musculature, one at right and the other at left, distant from the lung bases, hepatic vessels and from heterogeneous areas, in order to avoid artifacts. The arithmetic means of the three hepatic measurements and two muscular measurements were obtained, and the ratio between means was calculated, corresponding to the signal intensity ratio (SIR). The SIR was utilized, instead of the signal intensity in the liver alone, as the SIR considers the usual decrease in signal intensity that normally occurs as the echo time elapses, and because of the need for a reference tissue, the paravertebral musculature, that usually does not undergo iron deposition. Based on the SIR, the estimated hepatic iron concentration (EHIC) was obtained by means of the formula:

$$e^{[5.808 - (0.877 \times T2^*) - (1.518 \times PI)]}$$

where T2* is the SIR obtained on the sequence with TE = 14 ms, and PI is the SIR of the intermediate-weighted sequence.

A previous study indicates that such formula shows excellent correlation between SIR and hepatic iron concentration measured in hepatic tissue obtained by means of biopsy ($r = 0.937$)[8].

Means and standard deviations for quantitative variables and frequency distribution for proportional variables were utilized for data analysis. The patients were grouped according to two EHIC cut-off points and according to transfusional group (either SG or RG); the results were cross checked and compared with each other and between the groups. The groups comparison was made by means of the chi-square or Fisher's tests (proportional variables and discrete quantitative variables) and by means of the *t* test for independent samples or the Kruskal-Wallis test (continuous quantitative variables), considering 5% ($p < 0.05$) as significance level. The correlations between SIR and serum iron and serum ferritin levels were evaluated by means of the Pearson correlation coefficient (r).

The project of the present study was duly submitted and approved by the Committee for Ethics in Research of the responsible institution (CAE 0013.0.107.000-09), and the consent of the patients or parents/caregivers were expressed by means of the signature of a term of free and informed consent.

RESULTS

Thirty-two patients were submitted to MRI and had their serum ferritin levels measured. Among these patients, 23 (71.87%) were sporadically submitted to blood transfusion (SG).

Table 1 MRI sequences parameters†.

Sequence	TE (ms)	TR (ms)	SL (mm)	FA	FOV
GE T1-weighted	4	120	7	90°	370
GE IW	4	120	7	20°	370
GE T2*-weighted	9	120	7	20°	370
GE T2*-weighted	14‡	120	7	20°	370
GE T2*-weighted	21	120	7	20°	370
TSE T2-weighted	82	3650	5	—	380

† According to Alústiza et al.[8]. ‡ TE was modified from 14 ms to 13 ms, considering results obtained in a pilot-study. TE, echo time; TR, repetition time; SL, slice thickness; FA, flip angle; FOV, field of view; GE, gradient-echo; IW, intermediate weighting; TSE, turbo spin-echo.

There was a subtle predominance of the male gender, with 19/32 patients (59.37%). The mean patients' age was 14.21 ± 5.46 years. The mean serum ferritin value was 1,124.34 ± 644.26 μg/l, and serum iron value was 123.94 ± 45.12 μg/dl. The mean values for alanine transaminase and aspartate transaminase were 35.62 ± 27.82 U/l and 46.65 ± 23.12 U/l, respectively. The mean annual hemoglobin level was 8.20 ± 1.24 g/dl, and the total leukocyte count had a mean value of 12,009 ± 3,764/mm^3 and platelets, 370,853 ± 134,101/mm^3.

The patients were stratified according to iron overload (according to EHIC calculated from the MRI results) at two cut-off points – 40 μmol/g and 80 μmol/g – and the clinical and laboratory predictors of iron overload were evaluated. Figure 1 shows liver MR images of a patient with EHIC of 20.7 μmol/g (A) and 262.2 μmol/g (B).

Serum ferritin levels presented a mean value 58% higher in the group with iron overload, as 40 μmol/g was utilized as cut-off point, and 50% higher as the cut-off was 80 μmol/g, with statistically significant differences in both cases (p = 0.0001 and p = 0.0002, respectively). The "transfusional group" variable demonstrated to be predictive for overload, such an overload being greater in the group receiving regular transfusions (p = 0.0032 and p = 0.0234, respectively, for the EHIC cut-off points at 40 μmol/g and 80 μmol/g). The other variables did not present statistical significance (Table 2).

The correlation between the means of signal intensity ratios obtained from MRI and serum ferritin levels results, utilizing two reference groups as parameter (1,000 mg/dl and 500 mg/dl), was calculated and is shown on Table 3. It is observed that all the MRI sequences present lower signal intensity in the group with higher ferritin levels, for both cut-off points, a statistically significant result. Such a fact was not observed in cases where serum iron level was utilized as a reference.

The comparison of transfusional groups was based on the clinical and laboratory variables. The values that presented statistical difference were SIR of the MRIs, EHIC and serum ferritin level. The remaining clinical and laboratory variables did not present any statistically significant difference (Table 4).

Table 2 General patients' characteristics according to estimated hepatic iron concentration, demonstrated with two cut-off points.

	EHIC (μmol/g)					
	> 40	< 40	*p*	> 80	< 80	*p*
Age	14.42	13.9	0.8229	15.2	13.3	0.3047
Male	63 (12/19)	38.4 (5/13)	0.1766	57 (8/14)	50 (9/18)	0.4681
AST (U/l)	45.94	47.21	0.8734	46.5	46.7	0.9928
ALT (U/l)	39.31	29.50	0.4515	41.21	31.27	0.7812
Hb (g/dl)	8.41	7.90	0.2280	8.31	8.12	0.5857
Leuko (/mm^3)	12,257	11,932	0.3721	12,014	12,005	0.7947
Plat (/mm^3)	400,015	329,678	0.1451	378,378	365,000	0.7619
Iron (μg/dl)	121.20	127.73	0.6959	128.44	120.24	0.6203
Ferritin (μg/dl)	1,477.49	608.20	0.0001	1,560.07	785.44	0.0002
SG	52 (10/19)	100 (13/13)	0.0032	50 (7.14)	88.8 (16/18)	0.0234
RG	48 (9/19)	0 (0/134)		50 (7/14)	11.2 (2/18)	

EHIC, estimated hepatic iron concentration; AST, aspartate transaminase; ALT, alanine transaminase; Hb, annual mean hemoglobin; Leuko, annual mean leukogram; Plat, annual mean platelets; SG, sporadic transfusional group; RG, regular transfusional group.

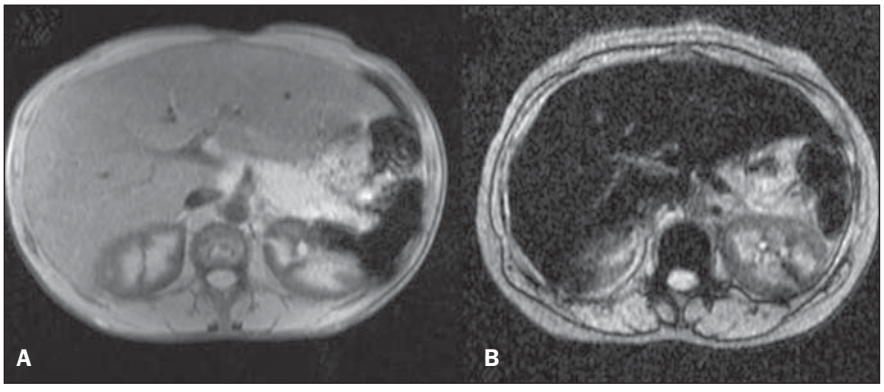

Figure 1. Abdominal MRI T2-weighted sequence* (TE = 13 ms). **A:** Patient with EHIC of 20.7 μmol/g. **B:** Patient with EHIC of 262.2 μmol/g. The noticeable difference in signal intensity between both livers is observed.

Table 3 MRI sequences weighting and their relationship with two groups (serum ferritin and serum iron).

	Ferritin (μg/dl)						Iron (μg/dl)		
Signal intensity	> 1.000	< 1.000	*p*	> 500	< 500	*p*	> 150	< 150	*p*
T1†	0.7233	1.2145	0.0017	0.7952	1.2386	0.0062	0.8729	0.9188	0.8082
IW‡	0.6433	1.0818	0.0005	0.7036	1.1171	0.0014	0.7486	0.8025	0.7618
T2* 9 ms§	0.3795	0.9509	0.0002	0.4472	1.0357	0.0008	0.5729	0.5929	0.9111
T2* 13 ms§	0.2971	0.9009	0.0001	0.3556	1.0371	0.0001	0.4714	0.5304	0.7448
T2* 21 ms§	0.2024	0.7309	0.0002	0.2332	0.9229	0.0001	0.3386	0.4075	0.6752

† T1-weighted sequence; ‡ intermediate weighted sequence; § gradient-echo sequences T2-weighted sequences with echo times of 9 ms, 13 ms and 21ms, respectively.

Table 4 Comparison among individuals in the group undergoing sporadic transfusions and individuals in the group with regular transfusions, according to all the studied variables.

	SG	RG	*p*
EHIC (µmol/g)	62.5176	196.1711	0.0001
T1	1.0544	0.4400	0.0001
IW	0.9552	0.3733	0.0001
T2* 9 ms	0.7196	0.2078	0.0006
T2* 13 ms	0.6384	0.1422	0.0006
T2* 21 ms	0.4844	0.1211	0.0105
Ferritin (µg/l)	962.78	1,537.22	0.0249
Iron (µg/dl)	129.05	111.46	0.3297
AST (U/l)	44.0	53.44	0.4941
ALT (U/l)	29.69	50,77	0.1596
Leukogram (mean/year)	12,237.08	11,466.66	0.6047
Hemoglobin (mean/year)	8.22	8.21	0.9067
Platelets (mean/year)	369,625.00	391,033.33	0.6059
Age	14.26	14.11	0.7548
Male patients	43.5% (10/23)	77.8% (7/9)	0.1242
Female patients	56.5% (13/23)	22.2% (2/9)	

SG, sporadic transfusional group; RG, regular transfusional group; EHIC, estimated hepatic iron concentration; T1, signal intensity ratio (SIR) of T1-weighted sequence; IW, SIR of the intermediate weighted sequence; T2*, SIR of the gradient-echo sequences T2*-weighted with echo times of 9 ms, 13 ms and 21 ms, respectively; AST, aspartate transaminase; ALT, alanine transaminase.

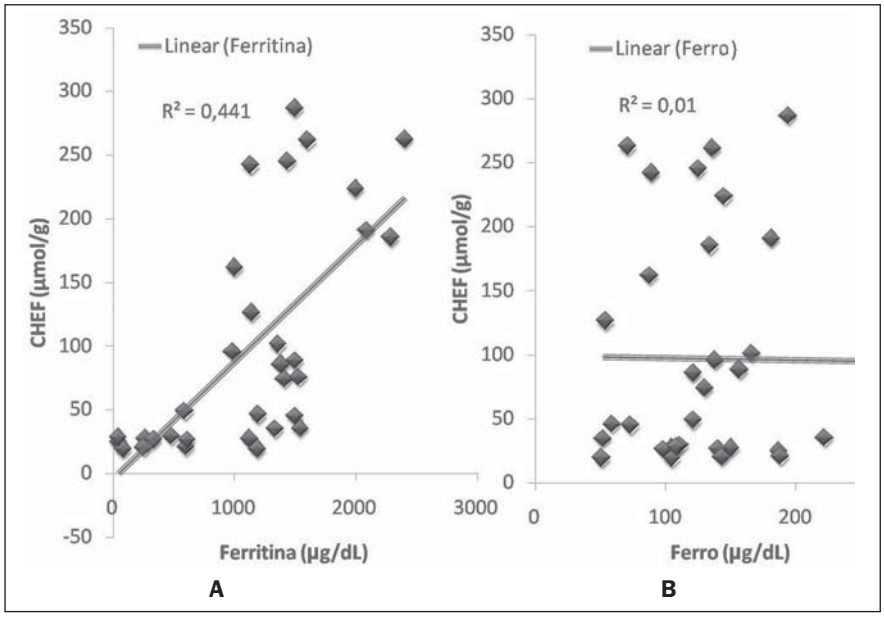

Figure 2. Correlation between EHIC and serum ferritin and iron levels. **A:** Correlation between serum ferritin levels and EHIC (r = 0.44). **B:** Correlation between serum iron levels and EHIC (r = 0.01).

Figure 2 represents the correlations between EHIC and serum ferritin (A) and iron (B) levels. Serum ferritin levels did not present good linear correlation with EHIC, with a Pearson correlation coefficient equal to 0.44. The correlation with serum iron level was even worse than that of serum ferritin level (r = 0.01).

DISCUSSION

An accurate and reproducible method for detection, evaluation and stratification of transfusion iron overload is a very useful tool in the clinical follow-up of patients with hemolytic anemia. The present study compared, in SCA patients, two strategies which are currently utilized for such purpose: serum ferritin test and MRI. The cut-off point for serum ferritin levels from which one can consider that tissue damage has already occurred is still to be determined: certainly, in cases where the values are > 1.000 mg/dl, the damages to organs and tissues have already occurred and some authors defend that the utilization of chelating agents be started at levels < 500 mg/dl[3]. In individuals with no risk factors for iron overload, it is expected the EHIC are below 20 µmol/g. For those with chronic hemolytic anemia, tissue damage seems to occur between 40 µmol/g and 80 µmol/g[4,5].

According to a previous study, serum ferritin levels do not present good correlation with the hepatic iron concentration[11]. However, other sources indicate a linear correlation between serum ferritin levels and total estimated body iron by means of the evaluation of tissue iron concentration in liver biopsy specimen[3,9,12]. However, it is known that the quantification of serum ferritin may be influenced by a number of factors, such as gender, infections, hepatopathies and cancer[3,13,14]. For that reason, its serial measurement is more accurate than the isolated measurement[13,14].

Angulo et al.[13] have not demonstrated linear relationship between mean serum ferritin levels in four years and the EHIC obtained by MRI, a finding that is in agreement with the present study.

Based on the present results, it is possible to observe that the only clinical and laboratory predictive variables for transfusion iron overload are serum ferritin levels and transfusional group, which is in agreement with reports in the literature[3,12]. Hepatic enzymes did not demonstrate to be a good iron overload indicator, which has already been previously observed[12]. In the present study, no other variable could be utilized as an iron overload predictor, independently from the utilized cut-off point.

It is important to highlight that the liver is the main iron storage organ, and the first to be injured, however the lesion of other organs, such as kidneys, pancreas, lungs, endocrine glands and heart, may decisively contribute to the death of patients with SCA[3,12,13,15,16].

The long term effects of chronic iron overload have been more extensively de-

scribed in patients with thalassemia, and SCA particularities may not allow the simple transfer of findings, among them the persistent activation of the inflammatory response cascade, as it modifies the serum ferritin concentration. Thus, in SCA patients, such variable is not a good indicator of iron overload in the organs. On the other hand, the serial imaging evaluation of a sentinel organ – the liver – may yield more reproducible data.

By comparing the iron-overload intensity between the patient groups submitted to two different transfusion regimens, it is possible to conclude that the group undergoing regular transfusions is more subject to the overload complications as, on average, such group presents three times the EHIC as compared with the group undergoing sporadic transfusions. Such result is in agreement with data in the literature, as the number of transfusions is already a well established predictive factor for iron overload[3,12]. The other variables that are predictive of overload (signal intensity and serum ferritin levels) were also altered in that group. Therefore, the RG patients must be closely followed-up particularly for the diagnosis and treatment of transfusional iron overload.

The categorization of patients according to the transfusional profile and the confirmation that the group under regular transfusions presents more consistent signs of hepatic iron overload is justified because indicates a group of patients as priority candidates for MRI evaluation as soon as they receive the indication and start receiving regular transfusions. The periodicity of such evaluation could not be determined in the present study, because of its cross-sectional characteristic. This is a limitation of the study, which for the same reason did not sequentially evaluate the serum ferritin concentration. In spite of such fact, the present study results indicate that the assessment of hepatic iron overload by means of MRI in SCA patients submitted to regular erythrocyte transfusion may be useful to determining preemptive therapeutic interventions based on the utilization of iron chelating agents, with a view on the preservation of the functions of several organs and systems.

CONCLUSION

The EHIC obtained by means of MRI was efficient in the estimation of hepatic iron overload in SCA patients undergoing regular red blood cell transfusion regimen.

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