Liver Biopsy Results in Patients with Sickle Cell Disease on Chronic Transfusions: Poor Correlation With Ferritin Levels

Lina B. Karam, MD,1 Deborah Disco, RN,2 Sherron M. Jackson, MD,2 David Lewin, MD,3 Virgil Mckie, MD,4 Robert D. Baker, MD,5 Susan S. Baker, MD,5 Joseph H. Laver, MD,6 Paul J. Nietert, PhD,7 and Miguel R. Abboud, MD8*

INTRODUCTION

Chronic transfusions are effective in preventing stroke and other complications of sickle cell disease. The aim of this study was to determine whether serum ferritin levels correlated with liver iron content in sickle cell patients on chronic transfusion. Forty-four liver biopsy specimens from 38 patients with homozygous sickle cell anemia (HbSS) and one patient with sickle thalassemia receiving chronic transfusions were studied. Five patients underwent a second liver biopsy for follow up. Three ferritin measurements were used to calculate a mean for each patient. The association between serum ferritin levels and liver iron quantitation was measured using the Spearman rank correlation, and sensitivity and specificity were determined for selected threshold values of serum ferritin. Serum ferritin levels ranged from 515 to 6076 ng/ml, liver iron concentration ranged from 1.8 to 67.97 mg/g dry weight. The amount of iron per gram liver dry weight was moderately correlated with serum ferritin values (r = 0.46). The correlation of duration of transfusion with serum ferritin (r = 0.40) and with liver iron content (r = 0.41) also indicated moderate correlation. Liver biopsy results led to changes in the management after 29/44 (66%) of the biopsies, Serum ferritin ≥2500 ng/ml predicted high liver iron content (>7 mg/g), with a sensitivity of 62.5% and a specificity of 77.8%. We found a poor correlation between serum ferritin levels and liver iron content (LIC). Despite being on chelation therapy, many patients on chronic transfusion had high levels of liver iron. Measurement of LIC is highly recommended in these patients. Pediatr Blood Cancer 2008;50:62–65. © 2007 Wiley-Liss, Inc.

Key words: sickle cell disease; liver biopsy; hemochromatosis; iron; ferritin; blood transfusion

MATERIALS AND METHODS

Thirty-eight patients with homozygous sickle cell anemia (HbSS) and one patient with sickle thalassemia (HbSP) were studied. Medical records of these patients were reviewed for the following information: hemoglobin electrophoresis, transfusion history, chelation therapy, ferritin levels, plasma vitamin C levels, hepatitis B and C serology, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, bilirubin direct and indirect, total protein and albumin. Serum ferritin concentrations were determined by automated microparticle enzyme immunoassay. Three ferritin measurements were used to calculate a mean level for each patient. A serum ferritin level was obtained at the time of the liver biopsy. The time interval between liver biopsy and other serum ferritin levels ranged from 0 to 12 months (mean of 6 months).

Informed consent was obtained from each patient or parent prior to each procedure. The liver tissues were collected during percutaneous liver biopsy in thirty cases. Fourteen patients had wedge liver biopsy; 11 during cholecystectomy, three during splenectomy. Specimens were evaluated histologically by one pathologist. Iron quantitation was performed in 40 of the 44 liver biopsies, at Mayo Clinical Laboratories (Rochester, MN). All the

© 2007 Wiley-Liss, Inc.
DOI 10.1002/pbc.21215
work-up done on these patients was done as part of direct patient care. The liver biopsies were performed to adjust iron chelation therapy in these patients and not as part of the study. This retrospective study was reviewed by the Institutional Review Board at the Medical University of South Carolina and deemed exempted from informed consent.

The association between serum ferritin levels (both the mean and the one closest in time to the liver biopsy) and iron quantitation was measured using Spearman rank correlation coefficients, as both the serum ferritin and iron quantitation were not normally distributed. Since iron accumulates in tissues with age, the hepatic iron index (hepatic iron concentration/age) corrects for this normal process. Thus we also measured the correlation between serum ferritin and the hepatic iron index. In addition, the correlations between the iron measures and histological grade were calculated, and using Wilcoxon rank sum tests, serum ferritin and liver iron were compared between patients with stage 1 fibrosis and those with more advanced fibrosis.

Sensitivity and specificity were determined for serum ferritin, treating iron quantitation as the gold standard. Threshold values for serum ferritin and liver iron were 2500 ng/ml and 7 mg/g, respectively, as values above these cutoffs are traditionally treated as markers of increased risk of cardiac disease and early death [9].

RESULTS

There were 25 males and 14 females, ages 6–23 years, with a mean age of 12.7 years. None of the patients were positive for hepatitis C or had other evidence of viral hepatitis on serology. All patients had normal vitamin C levels. Patients had a mean ALT of 42.7/21.2 IU/l at the time of biopsy. Two patients had elevated ALT and the value was within two times the upper limit of normal. One patient had a liver biopsy because of abnormal liver enzymes and hepatomegaly on physical exam. One patient had a concomitant diagnosis of rheumatoid arthritis. All patients had a liver biopsy to evaluate iron content and decide on initiation or adequacy of chelation therapy. Five patients had a second liver biopsy as a follow-up 29.8/9.2 months after the first biopsy (range 19–38). One patient had received erratic transfusions and the liver biopsy was done to evaluate the need for chelation therapy.

Patients were on chronic transfusion for a mean of 41.27/40.7 months (range 9–188). Thirty-eight were transfused for the prevention of recurrent strokes. Patients transfused to prevent stroke recurrence were kept at HbS < 30%, the others were kept at HbS < 50%. Thirty-seven patients were on chelation therapy for 38.9 ± 27.3 months (range 6–106). Thirty-two patients were on chelation with subcutaneous (SC) deferoxamine (30–42 mg/kg daily, 5 days per week). Three patients who were noncompliant with SC administration were admitted to the hospital once per week or every 2 weeks to receive intravenous (IV) desferrioxamine. One patient was on partial exchange transfusion because of poor compliance with deferoxamine. Chronic transfusion was stopped in one patient because of refusal of desferrioxamine; he was then placed on hydroxyurea. Quantitative iron was moderately correlated with the months of transfusion (r = 0.41, P = 0.011). Liver iron content ranged from 1.8 to 67.97 mg/g dry weight with a mean of 15.27 mg/g dry weight.

Serum ferritin ranged from 515 to 6076 ng/ml with a mean of 2808 ± 1429 ng/ml. A moderate correlation (see Fig. 1) was observed between the amount of iron on liver biopsy and both the mean serum ferritin values (r = 0.46, P = 0.004) and the serum value closest in time to the biopsy (r = 0.41, P = 0.009). Mean serum ferritin was also moderately correlated with the duration of transfusion (r = 0.40, P = 0.013), as was the correlation between serum ferritin levels and hepatic iron index (r = 0.39, P = 0.014).

Fig. 1. Association between serum ferritin and liver iron. Mean refers to the mean of three samples of serum ferritin obtained in the 6–12 months before the biopsy and closest to the value obtained closest to the time of biopsy. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Levels of more than or equal to 7 mg/g of liver iron and 2500 ng/ml of serum ferritin were described as threshold values for a high iron (i.e., being at risk for cardiac disease and early death). Using these values, the sensitivity and specificity of the serum ferritin level were determined to be 62.5% and 77.8%, respectively.

Sixty six percent (29/44) of liver biopsies led to changes in chelation therapy. Four patients would not have been started on chelation based on serum ferritin levels but were started based on liver iron concentration. Eleven patients had an increase in the subcutaneous dose of deferoxamine. Partial exchange transfusion was started in seven patients because of evidence of significant iron overload. Another seven patients were either started on IV desferal or had changes made to it. In one patient after having an improvement of iron content on the follow-up liver biopsy, the IV deferoxamine dose was decreased.

All patients had hemosiderosis on liver biopsy. 10/44 (22.7%) liver biopsies had grade 4 and 17/44 (38.6%) had grade 3 iron staining. All patients had some degree of fibrosis; most of them had a grade 1 (66%). Liver iron content did not differ significantly ($P = 0.99$) between those with grade 1 fibrosis (mean ± SD: 15.3 ± 13.4) and those with a higher grade (mean ± SD: 14.3 ± 10.6). However, there was a strong correlation between liver iron and histological grading of hemosiderosis or fibrosis ($r = 0.78$, $P < 0.0001$). Three patients had portal fibrosis, despite being on transfusion for less than 2 years and having a serum ferritin <2000 ng/ml. Two of them were not receiving chelation. Liver biopsy demonstrated evidence of chronic hepatitis in one patient with normal liver enzymes. Another was found to have granulomatous hepatitis and coagulative necrosis. This patient had positive sputum cultures for *M. tuberculosis*. Iron staining did not correlate with Kupffer cell hyperplasia in histological specimens.

**DISCUSSION**

Patients with thalassemia major who are transfused from infancy develop iron overload. The target organs for damage as well as the level at which tissue iron needs to be maintained to prevent cardiac dysfunction and death have been well defined in this population. Similar data are not available for patients with sickle cell disease an increasing number of who are receiving chronic transfusions, mostly for primary and secondary stroke prevention. The tissue iron load in these patients has traditionally been evaluated by serum ferritin measurements. In heavily transfused patients with thalassemia and SCD, studies have demonstrated that serum ferritin values are a poor and misleading measure of tissue iron load [4,11–13]. In this study, we demonstrate that ferritin measurements could be inaccurate in determining tissue iron content in individual patients with sickle cell disease. Another finding was that in our patients the incidence of end organ dysfunction, particularly cardiac, was low despite LICs that have been associated with organ dysfunction in thalassemia. The reasons for this are not clear and further studies are needed to define tissue iron levels at which end organ dysfunction will develop in patients with SCD.

There are several possible explanations for the poor correlation between serum ferritin and LIC. Hemolysis and ineffective erythropoiesis may play a role in the variability of ferritin levels in these patients [4,11]. Furthermore, ferritin is an acute phase reactant and may be increased by fever, acute infection, chronic inflammation and liver disease. We attempted to eliminate the role of acute changes by averaging ferritin values over one year but still did not find a correlation with liver biopsy results. Ascorbate deficiency, another cause for variation in ferritin levels, was not common in our patients who had ascorbate levels measured frequently. Regardless of the cause, serum ferritin was a very poor predictor of body iron stores in patients with sickle cell disease and significant changes were made in the management of 66% our patients after liver biopsy and LIC results were obtained. These changes included, starting chelation in patients whose ferritin levels were low, and increasing the dose and/or intensity of chelation. This was important in patients with very high iron levels, associated with imminent organ dysfunction that required intravenous chelation.

Iron is unevenly distributed in the livers of β-thalassemia patients, and the iron content determined in a small liver fragment should be interpreted in caution [14]. Nonetheless, the use of needle biopsies in evaluating liver iron content has been validated [15]. In addition, liver biopsies provide a more accurate assessment of the extent of the liver injury, inflammation, fibrosis, and cirrhosis. In our patients the liver iron content did not correlate with fibrosis. Despite having a significant level of iron overload, most of our patients had a low degree of fibrosis. These findings suggest that patients with SCD have a different response to iron overload when compared to patients with thalassemia or hemochromatosis. Another possible explanation, however, could be that it is just a matter of time and patients with sickle cell disease eventually develop significant fibrosis and cirrhosis as a result of iron overload.

As increasing numbers of sickle cell patients are started on chronic transfusion regimens, a reliable method for evaluating body iron stores and monitoring chelation therapy is needed. Despite the obvious limitations multiple serial ferritin measurements offer a quick, inexpensive and non invasive method to follow patients with iron overload on chelation. Percutaneous liver biopsy is a common and safe procedure that is often performed at the bedside or in the outpatient department [16–26]. In our patients, percutaneous liver biopsy was not associated with complications and in a series of 1184 percutaneous liver biopsies in 501 thalassemic patients, the complication rate was 0.5% and no complication was fatal [10]. Nonetheless, liver biopsy remains an invasive procedure and may not be acceptable to many patients and practitioners. The availability of new magnetic resonance based techniques may replace liver biopsies for determination of liver iron content [27]. The new MRI techniques allow the non invasive quantitation of both liver and cardiac iron. These technologies have been validated in comparison to liver iron content from biopsies and are available at many centers.

In light of recent advances, patients with SCD at risk for iron overload should be followed with serial serum ferritin as well as cardiac and liver iron quantitation by MRI (T2,T2* and R2). Biopsies would then be reserved for a limited number of patients who have persistent liver dysfunction, to evaluate fibrosis and other histologic changes [27]. These MRI studies will allow for better patient management and perhaps clarify the difference in cardiac and liver iron accumulation in this patient population. Such data may also point to different approaches to chelation as new oral chelators are now available that may be more effective in removing cardiac iron than desferrioxamine [28].

**ACKNOWLEDGMENT**

The authors would like to thank Ms. Ruby Khoury, BS, for her expert editorial assistance.
REFERENCES


28. Koliagou A, Economides C, Eraclious E, et al. Low serum ferritin levels are misleading for detecting cardiac iron overload and increase the risk of cardiomyopathy in thalassaemic patients. The importance of cardiac iron overload monitoring using magnetic resonance imaging T2 and T2*. Hemoglobin 2006;30:219–227.