Serum Ferritin Compared with Other Indices of Iron Status in Children and Teenagers Undergoing Maintenance Hemodialysis

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To determine how best to assess iron status, I studied 12 young renal patients (ages 5.5 to 20 years) undergoing regular hemodialysis treatments. Iron balance was estimated by monitoring iron loss ascribable to blood loss during dialysis and diagnostic testing, and iron intake in the form of oral and intravenous blood supplements and blood transfusions. Traditional methods of evaluating iron status—measurement of hemoglobin, erythrocyte indices, reticulocyte count, iron, and transferrin—were compared with measurement of serum ferritin. The serum ferritin measurements provided superior information. In three cases this method was superior to visual assessment of bone marrow stained for iron.

Additional Keyphrases: chronic renal failure, anemia, iron-binding capacity, iron overload, monitoring results of therapy

Normocytic normochromic anemia is a well-known consequence of chronic renal failure. Etiologic possibilities include one or a combination of the following factors: diminished erythropoietic-stimulating factor resulting from renal parenchymal damage (1), removal of folate and other water-soluble vitamins during dialysis (2), shortened erythrocyte survival in uremic patients (3), blood loss related to the dialysis process and frequent blood studies (4, 5), occult blood loss (6), dietary deficiency of iron, protein-calorie malnutrition, surgical procedures, infection, and hypersplenism. In addition to adequate dialysis, supplementation with vitamins and iron is believed to be beneficial in treating this anemia, especially in the patient who is not transfused (7, 8). However, these measures are usually insufficient to prevent the emergence of symptoms of anemia, and periodic blood transfusions may be required.

Therapy with oral iron, transfusion, or both requires that an accurate and minimally invasive procedure for monitoring iron status be available. Routine methods for evaluating iron status, such as bone-marrow staining and measurements of iron concentration and iron-binding capacity in serum, have serious practical and theoretical limitations in the pediatric uremic patient. Because of the need for better methodology for evaluating iron status in children on hemodialysis, I undertook this investigation into the value of measuring serum ferritin.

Materials and Methods

Subjects

Twelve young persons (seven girls, five boys) with end-stage renal failure were studied. They received two to three dialysis treatments per week, each lasting 4 to 5 h. All dialyses were performed during the hours 0800 to 1400. Table 1 shows selected clinical data on these patients. The mean age of the patients at the time of study was 13 yr. The mean duration of treatment by dialysis was 18.2 months (range, 1 to 54 months). If primary renal involvement was suspected, the diagnosis was confirmed by renal biopsy.

All patients had complete physical examinations at monthly intervals, and stool was tested for occult blood loss at that time. Similarly, appropriate blood studies were obtained, to detect liver disease or coagulation deficits. None of the patients included in this study had any notable abnormalities in these respects. The amount of blood obtained for these and other routine tests was monitored.

Patients H, I, and J received buffy-coat-poor packed erythrocytes at a rate of 180, 480, and 800 mL per month, respectively, for a total period of 12 to 58 months; a fourth patient, G, had received only two transfusions at the time of the study. No other patients received transfusion therapy.

Additional treatments included administration of folic acid (1 mg/day), Myadec (a multivitamin preparation with minerals, containing 250 mg of ascorbic acid and 20 mg of iron), Fer-In-Sol capsules or elixir in dosages to give 2–6 mg/kg of body weight per day of elemental iron, intramuscular iron dextran (Imferon), phosphate-binding gels, and calcium and vitamin D supplements. Dietary iron was calculated on the basis of 24-h dietary recall, recorded by the mother after detailed instructions by a dietitian.

Apparatus

Drake-Willock dialysis machines, models 4219 and 4019, were used. "Viva-cell" dialyzers (surface area, 0.5 m² or 1.0 m²) with a volume capacity of 50 mL or 95 mL, respectively (B-D Drake-Willock, Portland, OR 79222) were used, equipped with pediatric blood lines, models 09-510 and 09-512, and adult lines, model 09-341, with a volume capacity of 40, 75, and 155 mL, respectively (Cobe Laboratories, Lakewood, CT 80215).

A Coulter Counter, model "S" (Coulter Electronics Inc., Hialeah, FL 33010), was used to measure erythrocyte indices and microhematocrits, and serum iron was measured with a spectrophotometer (Coleman Instruments, Maywood, IL 60153).
A rate nephelometer (Hyland Laboratories, Costa Mesa, CA 92626) was used for all light-scattering measurements. A gamma counter (Nuclear-Chicago, Des Plaines, IL 60018) was used in the radioimmunoassay of ferritin.

Reagents

Anti-human albumin and anti-human transferrin were obtained from Technicon Instruments Corp., Tarrytown, NY 10591. The radiolabeled anti-human ferritin was purchased from Ramco Laboratories Inc., Houston, TX 77088.

Procedures

To estimate the amount of blood trapped in the dialyzer and connecting lines, I rinsed the entire assembly with 200 mL of a 0.9 mol/L solution of NaCl, then rinsed it with air, dismantled the dialyzer, washed the dialysis membranes, and measured the hematocrit of the eluate. I computed the iron content of blood lost or blood received according to the following relationships: 3.4 mg of iron per gram of hemoglobin, and 220 g of hemoglobin per liter ofuffy-coat-poor packed erythrocytes (9).

Blood samples were drawn before and after dialysis, for measurement of serum iron, transferrin, albumin, and ferritin. The hemoglobin, hematocrit, absolute reticulocyte count, and erythrocyte indices were measured in the predialysis blood specimen.

Serum iron was measured by the method of O'Malley et al. (10). Transferrin and albumin were measured by an immunonephelometric method (11). Serum ferritin was measured by the radioimmunoassay method of Addison et al. (12), as modified by Miles et al. (13).

Results

Hematologic Data

The mean hemoglobin concentration of the group was 61 g/L with a range of 43 to 88 g/L, and the mean proportion of reticulocytes was 1.8%, with a range of 0.4 to 4.0%. Erythrocyte indices were measured several times in each patient. Mean (+SD) values for the group were as follows: mean erythrocyte volume, 88 ± 4.8 fl; erythrocyte hemoglobin, 30 ± 2 pg per cell; mean erythrocyte hemoglobin concentration, 340 ± 10 g/L. Only patient B had microcytic hypochromic indices. (Examination of peripheral blood smears confirmed the values given by mechanized erythrocyte analysis.)

Estimation of Iron Balance

Oral iron intake from dietary iron and iron preparations was estimated for each patient and adjusted by using a minimal absorption factor of 0.13 (14). To the estimated quantity of absorbed iron, the iron supplied by iron–dextran injection or by packed-erythrocyte transfusion was added where appropriate. The mean estimated daily iron acquired by nontransfused patients was 13.2 ± 8.3 mg/day; for the patients receiving transfusions, 25.1 ± 8.4 mg/day (Table 1).

In the nontransfused patients the daily amount of absorbed iron was 18-fold the estimated iron loss, whereas the chronically transfused patients had almost 28-fold more iron absorption than losses. Even with a positive iron balance of this magnitude, all patients were found to have laboratory results consistent with a diagnosis of normochromic normocytic anemia.

Estimated iron loss due to blood loss during dialysis and routine sampling for diagnostic tests is also shown (Table 1). The amount of blood remaining in the dialyzer and connecting lines after the blood was returned to the patient averaged 5 mL (range, 2–10 mL, depending on the size of the dialyzer and the type of blood line used); this accounted for a mean estimated iron loss of 1.97 mg per dialysis, or 0.76 ± 0.18 mg/day when blood losses from diagnostic studies were also considered.

In Vitro Assessment of Iron Status

Predialysis measurement of serum iron and transferrin gave the following results: patients A through D had a low serum iron concentration (range, 350–620 μg/L; normal reference range, 680–1390 μg/L); patients F, G, and H had serum iron concentrations within the reference limits (840–1220 μg/L); and patient E and patients I through L had increased concentrations of serum iron (1560–2030 μg/L) (Table 2). Of all

1 Because of the conservation of iron by the body, a measure of oral intake and blood loss was assumed to constitute a fair estimate of iron balance in these patients. No female patients were known to be menstruating during the period of investigation with the exception of patient D, who experienced mild menorrhagia.
patients studied, only patient D had the classical biochemical markers of iron deficiency anemia, including low iron, high transferrin, and low ferritin; the mean serum transferrin concentration for the remaining 11 patients was 2780 mg/L (reference range, 2650-4300 mg/L). Only patient D had a low transferrin saturation (Table 2).

I found no correlation between serum iron, transferrin, or transferrin saturation and duration of dialysis, oral iron therapy, or transfusion frequency.

Serum ferritin concentration ranged from 5 to 1060 µg/L (reference range, 7-140 µg/L, Table 2). The highest ferritin values were found in the three chronically transfused patients (H, I, and J). Serum ferritin concentrations increased during a five-month period in patients H, I, and J, which corresponded with continuing transfusion therapy (Table 2). During the same period, there were no measurable changes in transferrin or transferrin saturation. Examination of the clinical course of patients H and I showed that the transfusion frequency had accelerated eight months before the study in patient H (with development of hypersplenism) and patient I (after bilateral nephrectomy was performed five months before the study). Liver biopsy results in patients H and J were consistent with hemosiderosis. Bone-marrow biopsies from these three patients showed a normal or mild increase in staining for iron when stained with Prussian blue. For patient H a bone-marrow biopsy concurrent with the liver biopsy was interpreted as normal iron staining; this patient developed an unexplained pericarditis and died from complications after splenectomy two months later. At autopsy, she was found to have extensive iron deposition in the liver, heart, and spleen, which correlated well with the serum ferritin concentration measured before death. Results of bone-marrow studies in this patient were inconsistent with autopsy and serum ferritin results.

Although serum ferritin values did not correlate with the duration of hemodialysis treatment, the frequency of blood transfusion did correlate with a significant increase in serum ferritin.

Effect of Dialysis on Iron Studies

To avoid artifactual changes in concentrations, that could result from changes in blood volume produced by ultrafiltration during dialysis, the change in body weight and serum albumin concentration was measured before and after dialysis in 10 patients. Paired t-test analysis of the data showed no significant change in pre- and postdialysis body weight or in concentrations of serum albumin, transferrin, or ferritin. However, the serum iron concentration was significantly increased ($p < 0.05$) after dialysis in nine of these 10 patients, as assessed by chi-square analysis. The mean increase in serum iron concentration was 27%; there was no direct correlation between the concentration of serum iron before dialysis and the percentage increase in serum iron during dialysis.

Discussion

Before introduction of a quantitative assay for serum ferritin, bone-marrow staining for iron was the only clinically available method for direct assessment of iron stores. Because bone-marrow biopsy is an invasive, painful, and relatively inconvenient procedure, it is unsuitable for sequential evaluation of iron stores, particularly in uremic patients. Moreover, there is considerable disagreement as to the age at which bone-marrow staining for iron becomes a reliable indicator of body iron stores (15, 16), calling into question the validity of the method. Finally, bone-marrow staining relies on the subjective estimation of iron stores; the results are only qualitative. This point was well illustrated in a comparative study of 60 adult anemic patients with rheumatoid arthritis (17); there, reproducible assessment of iron stores by serum ferritin measurement was superior to visual assessment of iron stores from bone-marrow staining. However, other studies have shown a good correlation between serum ferritin concentrations and stainable iron stores in adults on chronic hemodialysis (18, 19).

Disadvantages of conventional chemical methods for assessing iron status, such as measurement of iron and iron-binding capacity, include the relatively large serum sample needed, iron contamination from inadequately processed glassware and reagents, and the significant diurnal variation of serum iron (20).

These drawbacks of conventional methods for evaluating iron status in hemodialysis patients stimulated my investigation into the measurement of serum ferritin as a more practical and more precise means of assessing both the iron...
stores and the need for iron therapy in children on chronic maintenance hemodialysis.

An estimate of the iron status based on iron intake and blood loss was compared with hematologic and biochemical results. The estimates of iron balance were based on several assumptions: (a) that there is a strict conservation of iron by the body; (b) that the gastrointestinal absorption of iron in patients with chronic renal failure on hemodialysis is at least 13% of ingested iron; and (c) that there is 3.4 mg of iron per gram of hemoglobin. We have not taken into account the iron lost in the urine of our patients, because hematuria was either negligible or absent. Similarly, other occult blood losses were determined to be too small to significantly alter the estimated iron balance. Blood loss from venipuncture site leakage is extremely variable and, in our experience, very small; similar consideration was given to dialyzer rupture, which occurred infrequently (7 ruptures for every 1000 dialyses). Thus, these sources of blood loss were not included in the calculations of iron loss.

A very surprising finding was the large net iron intake of our patients and the extent to which the iron needs were overestimated, both in transfused and nontransfused young people. However, this was not altogether unexpected, because the main guide to the use of iron supplements was the hemoglobin concentration, with little regard for the many other factors contributing to the anemia. Hence, without proper and regular monitoring of body iron stores, the risk of iron overload can be great, particularly in those patients who require frequent blood transfusions.

Despite the small number of children available for study, the liver biopsies of two patients with markedly increased concentrations of serum ferritin revealed hemosiderosis; note that these two patients would not have been suspected as having iron overload on the basis of hematologic values and bone-marrow evaluation.

Another unexpected finding was the increase in the postdialysis serum iron, which occurred in nine of 10 patients. To my knowledge, this has not been previously reported. The diurnal variation of serum iron in normal children has been shown to follow a reverse pattern during the same period of time (29). Pre- and postdialysis body weights and albumin, transferrin, and ferritin concentrations in serum were not altered in most of the patients, which indicates that volume contraction from ultrafiltration was not responsible for the increased concentration of serum iron. Breakdown of erythrocytes in the dialyzer and absorption of iron from food ingested during dialysis are tentative explanations for this increase in serum iron during dialysis.

In summary, these results indicate that classical hematologic and biochemical measurements do not adequately reflect the iron status of hemodialysis patients. No correlation was seen between length of dialysis, therapy with oral iron, or transfusion therapy and erythrocyte indices, iron, transferrin, or transferrin saturation. In contrast, the concentration of serum ferritin correlated with oral iron therapy (the nontransfused group had a higher ferritin concentration than the control group) and with chronic transfusion therapy (the transfused group had a serum ferritin concentration >700 µg/L). Above-normal serum ferritin values were consistent with organ biopsies that indicated hemosiderosis in two patients. Because of the potential risk of iron overload in chronically dialyzed children, I recommend sequential monitoring of the concentration of ferritin in serum.

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References