Relative Iron Deficiency in Hereditary Spherocytosis

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Seventy-three patients with hereditary spherocytosis (HS) (58 nonsplenectomized, 15 splenectomized) were studied to evaluate iron status and the adequacy of iron availability for erythropoiesis. Splenectomized patients, who had hemoglobin levels in the normal or upper normal range, had higher levels of serum iron, transferrin saturation, and serum ferritin than normal matched controls and normal zinc protoporphyrin (ZnPP) levels. On the contrary, nonsplenectomized patients presenting with mild to severe anemia had higher red cell ZnPP concentrations than both splenectomized subjects and matched normal controls. ZnPP in nonsplenectomized patients correlated inversely with Hb concentration, mean corpuscular volume (MCV), mean red cell hemoglobin concentration (MCHC), transferrin saturation, and serum iron, and directly with reticulocyte count. At multiple regression analysis only Hb concentration was a significant explanatory variable for high ZnPP. The authors conclude that a number of nonsplenectomized HS patients have relative iron deficiency primarily because of expansion of erythropoiesis caused by anemia.

Key words: erythrocyte protoporphyrin, serum ferritin, hemolytic anemia, erythropoiesis

INTRODUCTION

Hemolytic anemias cause erythroid hyperplasia and increased hemoglobin production by individual erythroid cells, thus increasing the iron requirement for erythropoiesis. In both experimental animals [1] and patients with red cell disorders, iron supply has been shown to become inadequate to meet the requirements of intense erythropoiesis (relative iron deficiency) [2–4]. Relative iron deficiency has been found to restrict the size of newly formed red blood cells and has been associated with a rise of red blood cell protoporphyrin.

Previous investigations in our laboratory [5,6] showed a reduced mean corpuscular volume (MCV) in more than 70% of patients with hereditary spherocytosis (HS) when a correction for reticulocytosis was made. The present study was performed to evaluate the adequacy of iron availability for erythropoiesis and, in particular, to ascertain if relative iron deficiency affects patients with HS and to determine its frequency.

MATERIALS AND METHODS

Subjects
Data was analyzed on HS patients examined at the Centro Trasfusionale e di Immunologia dei Trapianti of the Ospedale Maggiore Policlinico, Milano, from 1974 to 1985. The patient sample consisted of 73 cases, 32 females and 41 males, from 2 to 57 (median 19) years old. Fifteen patients had been splenectomized at least one year before the present investigation.

Hereditary spherocytosis was diagnosed on the basis of clinical history, red blood cell count, peripheral blood examination, reticulocyte count, autohemolysis test, erythrocyte osmotic fragility on fresh blood and after incubation, and acidified glycerol lysis test [7]. Complete family studies were performed in 55/70 cases, and HS was traced back in 48/55 cases. In all patients, direct antiglobulin test and screening for unstable and abnormal hemoglobins were negative.

The clinical conditions of all patients were stable at the time they were investigated. White blood cell count and ESR were measured to exclude inflammation, and serum albumin, alkaline phosphatase, aspartate aminotrans-

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The patients were compared with 73 normal subjects matched for sex, age, and, in most cases, for area of origin, with a negative clinical history and normal results at both physical examination and routine laboratory tests.

### Technical Procedures

The following laboratory tests were performed: hemoglobin concentration (Hb), mean corpuscular red cell volume (MCV), mean red cell hemoglobin concentration (MCHC), serum iron (SI), and transferrin saturation (TS) in all subjects, reticulocyte count in patients only, and red cell zinc protoporphyrin (ZnPP) level and serum ferritin (SF) in 43 patients and all controls.

Blood counts were performed on an ELT-800 automatic cell counter (Ortho Diagnostic Systems, Westwood, MA).

Reticulocytes were counted after staining fresh blood samples with brilliant cresyl blue. Serum iron and total iron binding capacity were determined by a differential coulometric technique [8] using a Ferrochem model 3050 analyzer (Environmental Sciences Associated, Bedford, MA).

Serum ferritin concentration was assayed using an RIA kit (Lisophase, Lepetit, Milano, Italy). Red cell zinc protoporphyrin was determined on whole blood by direct fluorometry [9] using an ESA-4000 hematofluorometer (Environmental Sciences Associated) calibrated by a standard supplied by the manufacturer and expressed in μg/dl RBCs.

### Survey of the Effect of Reticulocytosis on ZnPP Measurement

Because attention has been drawn to the possible influence of reticulocyte count on ZnPP [10,11], the quantitative relation between this factor and ZnPP measurements was investigated.

Heparinized blood of five normal subjects and two HS patients was deprived of leukocytes and platelets by filtration on alpha cellulose-microcrystalline cellulose [12]. Washed red cells were then fractionated on Percoll gradients. Fractions of various density were brought to packed red cell volume (PCV) 0.40, and ZnPP and reticulocyte count were determined on each fraction. Hexokinase activity of the fractions was used as an indicator of the red cell age.

The values of ZnPP measurements in the various fractions had a coefficient of variation of 0.02. No relation was found between reticulocyte count, which decreased with increasing age of the cells, and the ZnPP level. Thus, we conclude that the reticulocyte count did not affect the ZnPP measurement.

### Statistical Analysis

Both the t-test and the Mann-Whitney nonparametric test for non-normal distributions (for SF only) were used to assess the significance of the difference between variables in two groups.

The Pearson correlation coefficient was used for a simple correlation study.

The Wilcoxon matched-pairs test was used to analyze differences between two groups of paired observations.

Relations between a dependent (predicted) variable and one or more independent (predictor) variables were investigated by linear multiple regression analysis; it was carried out by entering independent variables in the regresion function step by step and assessing the statistical significance of the introduction of the variable by the F-test. The Statistical Package for Social Sciences (SPSS) [13] and Generalized Linear Interactive Modeling (GLIM) [14] packages were used.

### RESULTS

#### Red Cell Indices and Iron Status Parameters

The patients’ hematological characteristics are shown in Table I. With respect to the controls, the HS patients (splenectomized and nonsplenectomized) showed a significantly lower Hb concentration and higher MCV, MCHC, TS, ZnPP, and SF.

Splenectomized and nonsplenectomized patients are considered separately in Table II. The two groups differed in Hb concentration, reticulocyte count, and ZnPP. In the splenectomized patients Hb concentration, MCV, SI, TS, and SF were higher than in the corresponding controls. The mean ZnPP level was normal, and no pa-

### Table I. Sex, Age, Red Cell Indices, and Iron Status Parameters of HS Patients (15 Splenectomized, 58 Nonsplenectomized) and Matched Controls (Means ± SD Except for SF, Which Is Expressed as Median and 25th–75th Centile Interval)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>HS Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>41/32</td>
<td>41/32</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23.5 ± 14.5</td>
<td>23.9 ± 14.9</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.7 ± 1.3</td>
<td>12.7 ± 2.9</td>
</tr>
<tr>
<td>Reticulocytes (10^9/l)</td>
<td>n.d.</td>
<td>335 ± 243</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>86.8 ± 3.3</td>
<td>88.1 ± 6.7</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.1 ± 1.02</td>
<td>33.4 ± 2.2</td>
</tr>
<tr>
<td>SI (μg/dl)</td>
<td>88.0 ± 29.4</td>
<td>96.1 ± 32.3</td>
</tr>
<tr>
<td>TS (μg/dl)</td>
<td>30.0 ± 11.4</td>
<td>35.8 ± 13.2</td>
</tr>
<tr>
<td>ZnPP (μg/dl RBCs)</td>
<td>45.8 ± 28.0</td>
<td>68.0 ± 42.1</td>
</tr>
<tr>
<td>SF (μg/l)</td>
<td>54 (31–80)</td>
<td>120 (50–300)</td>
</tr>
</tbody>
</table>

*Significant P values (Wilcoxon test for paired data) between HS patients and controls are given.

n.d., not determined.
TABLE II. Sex, Age, Red Cell Indices, and Iron Status Parameters in Splenectomized and Nonsplenectomized Patients and in Their Respective Matched Control Groups (Means ± SD Except for SF, Which Is Expressed as Median and 25th–75th Centile Interval)*

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 15)</th>
<th>Splenectomized HS patients (n = 15)</th>
<th>Nonsplenectomized HS patients (n = 58)</th>
<th>Controls (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>9/6</td>
<td>9/6</td>
<td>32/26</td>
<td>32/26</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>28.8 ± 15.6</td>
<td>29.0 ± 15.4</td>
<td>22.5 ± 14.6</td>
<td>22.3 ± 14.0</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.9 ± 1.2</td>
<td>15.8 ± 1.7</td>
<td>11.8 ± 2.6</td>
<td>13.6 ± 1.3</td>
</tr>
<tr>
<td>Reticulocytes (10⁹/l)</td>
<td>n.d.</td>
<td>116 ± 51</td>
<td>384 ± 242</td>
<td>n.d.</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87.1 ± 2.3</td>
<td>90.4 ± 4.9</td>
<td>87.4 ± 7.0</td>
<td>86.7 ± 3.5</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.2 ± 0.9</td>
<td>33.8 ± 1.5</td>
<td>33.3 ± 2.3</td>
<td>33.1 ± 1.0</td>
</tr>
<tr>
<td>SI (µg/dl)</td>
<td>78.8 ± 20.3</td>
<td>112 ± 33.1</td>
<td>92.4 ± 30.9</td>
<td>89.4 ± 29.4</td>
</tr>
<tr>
<td>TS (%)</td>
<td>27.3 ± 8.4</td>
<td>41.5 ± 9.1</td>
<td>34.5 ± 13.6</td>
<td>30.2 ± 11.5</td>
</tr>
<tr>
<td>ZnPP (µg/dl RBCs)</td>
<td>46.3 ± 32.2</td>
<td>52.1 ± 23.0</td>
<td>77.9 ± 41.9</td>
<td>43.0 ± 28.6</td>
</tr>
<tr>
<td>SF (µg/l)</td>
<td>61 (5–86)</td>
<td>145 (50–320)</td>
<td>120 (50–370)</td>
<td>52 (35–110)</td>
</tr>
</tbody>
</table>

*Significant P values (t-test and Mann-Whitney test for SF only) between nonsplenectomized and splenectomized patients and significant P values (Wilcoxon test for paired data) between patients and controls are given.

n.d., not determined.

Patient had a ZnPP value above the 90th percentile of the respective control group (84 µg/dl RBCs).

Compared with the controls, the nonsplenectomized patients were more anemic, their MCV value was not significantly different despite the high reticulocyte count, and SF was significantly higher. SI and TS were marginally increased, but the difference was not statistically significant. Even though the above parameters suggested normal-increased iron stores, the patients showed a higher mean ZnPP level than did the controls. In fact, 45.4% of the nonsplenectomized HS patients had a ZnPP level above the 90th percentile of the control group (71 µg/dl RBCs).

As this study was concerned with the evaluation of iron supply for erythropoiesis, only the nonsplenectomized patient group, which had higher levels of ZnPP than did the controls, was considered in the subsequent analysis.

Hb and iron status parameters in these patients, divided according to sex, are reported in Figure 1. The mean Hb level was lower (11.1 vs. 12.5 g/dl, P < .05) and ZnPP higher (94.8 vs. 68.2 µg/dl RBCs, P < .05) in the females than in the males. One female patient with high ZnPP values had TS of under 15%, whereas none had SI of under 30 µg/dl or SF of under 12 µg/l.

Analysis of Nonsplenectomized Patients with High and Low ZnPP Values

Table III shows age, Hb level, red cell indices, and iron status parameters in nonsplenectomized patients subdivided in two subgroups according to ZnPP concentration (low ZnPP < 71 µg/dl RBCs, high ZnPP > 71 µg/dl RBCs). Patients with high ZnPP value had lower Hb concentration, SI, and TS and higher reticulocyte count.

Linear and Multiple Regression Analysis

In the nonsplenectomized patients, Hb decrease was associated with a rise in reticulocyte count (r = -0.47, P < .05), thus demonstrating that erythropoiesis was physiologically controlled. The regression lines between reticulocyte count and Hb concentration did not differ significantly in patients with high and low ZnPP level.

The increase of ZnPP value was closely associated with a decrease of Hb and SI and with an increase of reticulocyte count (Fig. 2). A marginally significant inverse relation was found between ZnPP and MCV, MCHC, and TS.

A multiple regression analysis with ZnPP as dependent variable showed that in HS patients Hb concentration alone accounted for 30% of the variance of ZnPP, and significant improvement of the regression coefficient was not achieved when any of the other variables were included. Hb and sex together accounted for 57% of the ZnPP variance.

DISCUSSION

Relative iron deficiency refers to a condition in which iron stores are not decreased, but the availability of iron from tissues, primarily reticuloendothelial, for erythropoiesis is inadequate to meet the increased iron requirement.

Of the nonsplenectomized HS patients examined in the present study, 45% were found to have relative iron deficiency on the basis of increased ZnPP values. The "relative" nature of iron deficiency of our patients may be deduced from two considerations. First, the patients had normal or slightly increased body iron stores as evaluated by serum ferritin, and plasma iron transport was normal...
Fig. 1. Hemoglobin and iron status parameters of nonsplenectomized HS patients divided according to sex (♂, males, ♀, females). Serum ferritin is expressed as log scale. Horizontal bars represent mean ± SD, or median and 5th and 95th centiles (for SF only). Significant P values are also given.

### TABLE III. Sex, Age, Red Cell Indices, and Iron Status Parameters in Low- and High-ZnPP Nonsplenectomized HS Patients (Means ± SD Except for SF, Which Is Expressed as Median and 25th–75th Centile Interval)*

<table>
<thead>
<tr>
<th></th>
<th>Low ZnPP (&lt;71 µg/dl RBCs; n = 18)</th>
<th>High ZnPP (&gt;71 µg/dl RBCs; n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>13/5</td>
<td>8/7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>26.0 ± 13.8</td>
<td>18.8 ± 13.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.1 ± 1.9</td>
<td>11.2 ± 2.8</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>89.8 ± 3.7</td>
<td>85.8 ± 8.4</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.4 ± 0.8</td>
<td>31.7 ± 3.3</td>
</tr>
<tr>
<td>Reticulocytes (10³/fl)</td>
<td>262 ± 133</td>
<td>385 ± 208</td>
</tr>
<tr>
<td>SI (µg/dl)</td>
<td>95.3 ± 23.2</td>
<td>69.7 ± 17.8</td>
</tr>
<tr>
<td>TS (%)</td>
<td>35.9 ± 10.9</td>
<td>24.7 ± 9.1</td>
</tr>
<tr>
<td>SF (µg/l)</td>
<td>150 (53–400)</td>
<td>190 (52–560)</td>
</tr>
</tbody>
</table>

*Significant P values (t-test and Mann-Whitney test for SF only) between the two groups are given.

Iron status in congenital hemolytic anemias varies greatly. In some of them, such as thalassemia intermedia, congenital dyserythropoietic anemia, and congenital sideroblastic anemia, iron overload invariably develops from increased iron absorption [15,16]; in others, such as sickle cell anemia, body iron content is normal or decreased [11]; and in beta-thalassemia/HbE and in HbH as evaluated by serum iron and transferrin saturation. Second, at a multiple regression analysis, Hb level was the only variable that significantly correlated with ZnPP increase. This is consistent with the hypothesis that anemia-induced expansion of erythropoiesis is the only determinant of iron-deficient erythropoiesis. In agreement with this hypothesis, Langer et al. [2] have demonstrated that erythrocyte protoporphyrin levels are elevated in hemolytic anemia only when red cell production is five times higher than normal.

In the series presented here, the prevalence of relative iron deficiency was higher in the females than in the males. Thus, it is conceivable that a sex-related difference in body iron content may be an additional cause of defective iron availability for erythropoiesis. Moreover, age-adjusted MCV values were similar in normal controls and HS patients in spite of the high reticulocyte count in the latter. This, together with the slight inverse relation between MCV and ZnPP, suggests that a relatively low MCV is a feature of HS and a possible consequence of relative iron deficiency.
disease, relative iron deficiency has been found in a high proportion of cases [3]. As regards HS, a clinical syndrome of hemochromatosis has rarely been observed [17–22], and normal iron absorption measurements have been reported [23], in spite of increased erythropoiesis.

In conclusion, our results indicate that relative iron deficiency may be present in HS patients, particularly in females, reflecting the sex-related differences in body iron content. Further investigations are needed to define the causes of this phenomenon.

ACKNOWLEDGMENTS

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REFERENCES


