Hair iron content: possible marker to complement monitoring therapy of iron deficiency in patients with chronic inflammatory bowel diseases?

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Measurements of the concentrations of iron in hair from 10 patients with chronic inflammatory bowel diseases and from 10 healthy controls showed that the iron concentrations were significantly ($P < 0.05$) lower in patients before iron intake than in controls. Three weeks after beginning iron treatment, the hair iron concentrations were found to be significantly correlated ($r = 0.68; P < 0.05$) to reticulocyte counts. Changes in the hair iron concentrations were accompanied by similar changes in the concentrations of the markers most commonly used to diagnose and monitor iron deficiency. The results suggest that quantification of hair iron may be useful to complement evaluations of the body iron status.

INDEXING TERM: reticulocytes

Iron is ubiquitously distributed in nature and represents the most essential trace element in human organisms. An indispensable cofactor for several fundamental metabolic processes, aberrations in iron metabolism can be fatal for the organism. The iron balance in human body is vulnerable and, for its regulation, the organism produces transport and storage proteins (e.g., transferrin, haptoglobin, hemopexin, ferritin, and hemosiderin). The major functions of these proteins are the safe management, movement, and storage of iron.

Iron deficiency is the most frequent disorder of iron metabolism worldwide. The deficiency can be produced by an inadequate diet, abnormal absorption, and increased iron demands, or chronic blood loss. Iron excess can also arise, which may predispose the subject to infection [1, 2] by deleting its iron-withholding defenses and stimulating microbial growth. Correlations between the increase of the body iron concentration and increased risk of cancers have been reported [1, 3, 4]. Measurement of hematological and biochemical markers (blood count, serum iron, ferritin, and transferrin) allows assessment of the iron status. To our knowledge, however, no attempt has been made to include the measurement of hair iron in an evaluation of body iron. The aim of this study was to extend the assessment of the iron balance by including the measurement of hair iron concentration. We also investigated the potential value of this measurement as an aid in diagnosis and therapeutic monitoring in patients with chronic inflammatory bowel diseases.

Materials and Methods

The study group consisted of 10 ambulatory patients with Crohn disease or ulcerative colitis, recruited from the University Hospital Freiburg (Germany). The inclusion criteria were a clinical and histological diagnosis of a chronic inflammatory bowel disorder (Crohn disease or ulcerative colitis) associated with iron deficiency, and no previous (during the preceding 3 months) or current iron therapy.

All patients were fully informed about the study and informed consent was obtained from all subjects. Hair samples from 10 healthy volunteers were also analyzed. For both groups, specimens 2–3 cm long were cut with stainless steel scissors from the part closest to the scalp in the suboccipital region of the head. None of the subjects had undergone cosmetic hair treatment (bleaching, dyeing, or permanent waving) other than washing with shampoo at home during the previous 3 months.

Iron-deficient patients were treated with effervescent tablets (from Beiersdorf Corp., Hamburg, Germany) containing 695 mg of iron(II) gluconate (corresponding to 80.5 mg of iron), one tablet daily. The patients were instructed to dissolve the drug in

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a glass of water and to drink this ~30 min before breakfast. The
treatment was continued for 6 weeks, and patients were invited
to present themselves every 3 weeks at the hospital for follow-up
visits. After physical examination, blood and hair samples were
obtained from the patients.

There was no special need to wash or shampoo the hair
before cutting it, because the samples were washed before
analysis. Hair samples were washed first with a detergent
solution (10 mL/L 7X-o-Matic; Serva, Heidelberg, Germany)
and then three times with distilled water. To free the samples
from lipids, we also washed the hairs once with 5 mL of acetone.
After drying, we took 5-10 mg of weighed sample and wet-ashed
it with 0.5 mL of 70% nitric acid at 100 °C for 1 to 2 min. The
liquid was evaporated from the digest under a stream of nitrogen
at 100 °C, and the residue was mixed with 5 mL of 0.2% nitric
acid. Further heating at 100 °C for 5 to 10 min yielded a clear
solution suitable for analysis.

PROCEDURES

Assays. Iron concentrations (µg/g of hair) were determined by
atomic absorption spectrometry with a Model 1100B instrument
(Perkin-Elmer, Überlingen, Germany). Serum concentrations
of iron, ferritin, and transferrin and reticulocyte counts, hemat-
ocrit, and hemoglobin concentrations were measured by routine
methods.

Analytical evaluation. A hair sample from the same subject was
cut into 2- to 5-mm pieces and aliquoted into five portions of
equal weight for the recovery study. Before digestion, we added
to each hair sample different concentrations of iron solutions
(5-50 µg/g dry weight). To assess assay precision, we analyzed
several times hair samples from five healthy individuals (weighed
portions of 2- to 5-mm pieces). We assayed four replicates of
each of the five hair samples on five different days over a 10-day
period.

Statistical analysis. Results are given as mean ± SD. Student’s
t-test was used to test the level of significance of differences
between sets of data before and after treatment. A significant
difference was defined as P <0.05. The relation between two
variables was assessed by correlation analysis and by linear
regression.

Results

Analytical recovery of various concentrations of iron added to
hair homogenates from the same subject ranged from 91% to
103%. CVs ranged from 8% to 10%. The mean ± SD iron
content of hair from 10 healthy subjects was 130.7 ± 59.6 µg/g
(range, 47.6–213.0 µg/g). Before treatment, serum iron in
patients ranged from 110 to 490 µg/L, and the concentration of
iron in hair varied from 4.4 to 66 µg/g (mean ± SD, 29.0 ± 20.4
µg/g). The differences in hair iron concentrations between
healthy individuals and patients were statistically significant
(130.7 µg/g vs 29.0 µg/g, P <0.001).

The correlations between hair iron concentration and the

| Table 1. Correlation (r) of hair iron concentrations with
commonly used markers for assessing body iron status. |
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* Statistically significant at P <0.05.

other markers examined were consistently negative, except for
the reticulocyte count and transferrin saturation (Table 1). The
relation between serum transferrin and hair iron was statistically
significant (r = −0.73, y = −0.93x + 431.15) by 3 weeks after
beginning treatment. The relation between hair iron and trans-

Fig. 1. (Top) Relation between hair iron (µg) and serum iron concentra-
tions (x) in patients with chronic inflammatory bowel disease (CIBD)
before the start of iron therapy: y = −1.05 (SE 0.40) x + 62.18 (SE
13.40), r = −0.68 (P = 0.025); (bottom) relation between hair iron
concentration (y) and reticulocyte counts (x) in CIBD patients 3 weeks
after institution of iron substitution: y = 8.13 (SE 3.07) x − 17.50 (SE
76.57), r = 0.683 (P = 0.029).
ferrin saturation was positive ($r = 0.69, y = 3.44x + 27.52$). Before treatment with iron, a significant inverse relationship (Fig. 1, top) was found between hair iron ($y$) and serum iron ($x$), the regression equation being $y = -1.05x + 62.18$ ($r = -0.68, P < 0.05$). Serum iron and ferritin increased during the treatment period (Fig. 2). In 80% of the patients, the reticulocyte count, the transferrin saturation, and the hair iron content reached a maximum 3 weeks after beginning the iron supplement. This represented an absolute mean increase in reticulocyte count of 0.9%, which was statistically significant ($P < 0.001$). The absolute mean increase in hair iron concentration was 40.0 μg/g ($P < 0.001$), and transferrin saturation increased from 6.4% to 16.5%. As shown in Figs. 1 (bottom) and 3, changes in reticulocyte count ($x$) were associated with similar changes in hair iron concentration ($y$). After 3 weeks of iron treatment, the correlation between these two markers was significant (Fig. 1, bottom): $y = 8.13 x - 17.50$ ($r = 0.683, P < 0.29$). A decrease in the reticulocyte count and a slight increase in hemoglobin concentration were observed after 6 weeks of treatment.

**Discussion**

Few data have been reported supporting the use of hair analysis in clinical medicine. The major objections [5–8] to measuring trace elements in hair are: variations across the scalp, due to the different lengths of hair involved; environmental contamination; lack of correlation between concentrations of trace elements in hair and the values in other organs; and the absence of clearly defined reference ranges.

The use of hair analysis [9–16] has been restricted mainly to comparisons of different populations (e.g., measures of environmental exposure to heavy metals). Some investigators [17, 18], however, have examined trace elements in human hair for evaluating mineral nutritional status and for cosmetic uses. A few attempts have been made to examine both hair and blood samples from patients to monitor the course of a disease. Cystic fibrosis [19] is associated with high concentrations of sodium and chloride in hair, but hair analysis has not been used to diagnose this disease. Functional disorders have been reported in children with low concentrations of zinc in hair [18]. Data in the literature are conflicting and do not support at all the assumption that hair zinc concentration may be used as an index.
of the body zinc content. Epstein et al. examined 11 patients with primary biliary cirrhosis and found no high concentration of copper in hair [20]. Wang et al. [21] reported that trace element concentrations in hair reflected the clinical stages in blackfoot disease. However, few data are reported with regard to hair iron [22, 23], probably because well-proven assays are already available for assessing iron status.

The range of 48.0 to 213.0 μg/g for hair iron concentrations found in our healthy control group is in agreement with most of the previously reported values (Table 2). Moreover, these concentrations were significantly higher in healthy controls than in patients. Before therapy there was an inverse correlation between hair iron and serum iron values. This is in agreement with the hypothesis that hair concentrations of trace elements reflect the changes in element intake over a longer period, whereas plasma element values provide a record of more-recent intake. Therefore, the low concentrations of hair iron found in our patients may be associated with the iron deficiency. Then, during treatment, increases in hair iron were associated with increased plasma iron, ferritin, transferrin saturation, and reticulocyte count. By 3 weeks after the beginning of therapy, the mean hair and serum iron concentrations were significantly higher than the initial values, and reticulocyte counts now correlated significantly with hair iron concentrations.

The statistically significant relationship between hair iron concentration, reticulocyte count, and transferrin saturation is the most important and surprising finding in this study. This suggests that hair iron concentration very likely depends on both the bioavailability of iron and the extent of erythropoiesis. Hair iron concentration seems to be affected in advanced stages of iron deficiency but is less sensitive shortly after the onset of iron depletion. This hypothesis could be verified by comparing hair iron concentration with the serum concentration of transferrin receptor, which is considered to increase after the onset of iron deficiency [24].

The observed inverse relationships between hair iron concentration and the most widely used indicators of body iron status support the view that the time scales reflected by blood and hair are different [8]. Such observations have not been previously reported, except that Wang et al. [21] showed that the hair iron concentration of patients with blackfoot disease decreased as the clinical stages progressed. Their data and ours suggest that hair iron concentration may provide a short-term index as well as a long-term record of body iron status and may thus be useful for monitoring the course of the iron deficiency. In addition, because iron metabolism is regulated by the amount of iron absorbed rather than by the amount excreted, hair iron could be a useful complementary marker [25] in the assessment of body iron status. However, to confirm the clinical value of hair iron concentration will require extended studies that include patients with real iron deficiency, with increased erythropoiesis, and with anemia associated with ineffective erythropoiesis.

### References