
Minerals in Hair, Serum, and Urine of Healthy and Anemic Black Children

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Synopsis

Hair mineral analysis can be used as a reliable screening test for heavy metals, but it is not an

established method for defining nutritional and disease states. Wide variation in test results is a major problem in utilizing the technique for clinical purposes. Better reference values are needed, especially for children, as well as information about how hair mineral values correlate with body fluid values.

A total of 48 black children were studied. Of these, 20 were normal children, ages 1 to 17; 12 were normal infants, ages 5 weeks to 12 months; 3 were children with iron overload; 7 had iron deficiency anemia; and 6 had thalassemia trait. There were in all 17 boys and 31 girls. The distribution of 15 minerals in hair, serum, and urine samples was determined by energy dispersive X-ray fluorescence. Mineral concentrations from the normal children were compared with concentrations obtained from the children with iron overload, iron deficiency anemia, and thalassemia trait. Statistical analysis revealed no significant differences among any of the groups. Mineral concentrations from the normal infants and children may be useful as reference values.

The analysis of hair iron as a valid screening test for body iron status in children is not supported by our data.

HAIR ANALYSIS can be employed as a test for intoxication, the ingestion of drugs, or for contamination with lead, arsenic, cadmium, and mercury (1,2), but its usefulness for defining disease or nutritional status remains to be defined (3-5). Unfortunately, hair analysis has been used inappropriately in many clinical situations (6-9).

One major problem associated with analysis of hair minerals is that values reported for healthy persons have varied widely, probably because of methods of sampling, sample preparation, alterations caused by shampoos and hair treatment, and other forms of external contamination. Possible contamination from the atmosphere and various other sources may make mineral content inconsistent throughout the length of a strand of hair. Another problem is the need for better reference values, especially for children. Finally, for many of the minerals, concentrations in hair have not been

shown to correlate with concentrations in serum and other body fluids. Some minerals are known to compete for absorption and transport sites, so that a change in concentration of one mineral may cause changes in the concentrations of other minerals.

There are, on the other hand, a number of advantages associated with hair analysis. Hair samples can be obtained quickly and easily, without discomfort to the subject, and without the need for special equipment. Samples remain stable with no special requirement for storage conditions. Techniques used for analysis, including atomic absorption, neutron activation, and X-ray fluorescence, are sensitive. Hair analysis, if shown to be reliable and reproducible, may in the future become a useful screening procedure for selected clinical conditions. In a large study of the distribution of trace minerals in human hair reported recently,

'There was no significant difference between the concentrations of iron from the hair of our study children with iron overload and those with iron deficiency anemia . . .'

Sky-Peck (10) has helped to establish normal ranges by examining the differences associated with age, sex, and race, as well as hair color, hair treatment, and environmental exposure.

The aims of our study were to establish normal values for a number of minerals in hair, serum, and urine from a group of healthy black children and to compare these concentrations with those for children with iron overload, iron deficiency anemia, and thalassemia trait. We were especially intrigued by the possibility that hair iron could be a potentially useful screening test if levels present in various metabolic states were found to differ significantly from normal levels.

Materials and Methods

Subjects. A total of 51 black children were recruited from the Howard University Hospital pediatric service. Three were known to have hemolytic disorders with iron overload. The other 48 were randomly selected from the pediatric clinics, where they were being seen for routine examinations.

The study was approved by the Howard University Institutional Review Board. Informed consent was obtained for each child from a parent or guardian, and a questionnaire was completed. Information obtained from the questionnaire included the type of shampoo used, date of the most recent shampoo, any substance or substances applied to the hair after shampooing, hair processing—such as straightening or permanent waving—and the zip code of the child's address. The hair content of lead and strontium have been shown to vary because of contamination according to residence by zip code (10,11).

Sampling techniques. Hair samples were obtained by clipping 10 or more strands, each strand at least one inch in length, close to the scalp. Hair samples were stored in polyethylene zip-locked bags. A blood sample was collected by venipuncture and divided into three aliquots. A complete blood count

(CBC), hemoglobin electrophoresis, and glucose-6-phosphate-dehydrogenase (G6PD) screening test were performed on one aliquot. The CBC, carried out on an electronic blood cell counter, included a red blood cell count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC). Hemoglobin electrophoresis, blood lead, and free erythrocyte protoporphyrin (FEP) studies were carried out on the second aliquot. The third aliquot was centrifuged, the red blood cells discarded, and the serum frozen and stored for later analysis of minerals. If available, an aliquot from a single random urine specimen was frozen and stored for later analysis.

Sample preparation and analysis. Hair samples were washed with a mild anionic detergent (1 percent sodium lauryl sulfate), distilled water, isopropanol, distilled water again, and air dried in a laminar flow hood. The strands of hair were mounted in the sample vacuum chamber of the energy dispersive X-ray fluorescence (EDXRF) system (A) and analyzed. The quantitative EDXRF system, which has been described by Sky-Peck and Joseph (12), identified and quantified 15 elements: calcium (CA), chromium (CR), manganese (MN), iron (FE), nickel (NI), copper (CU), zinc (ZN), selenium (SE), bromine (BR), rubidium (RB), strontium (SR), lead (PB), mercury (HG), sulfur (S), and arsenic (AS). Standard solutions of the elements were analyzed for comparison, and the effects of background radiation scatter were subtracted by computer prior to the final reading.

Definition of anemia. Children under 12 years with an HB below 11 grams per deciliter (gm per dl) and HCT below 33 percent and children 12 years and older with an HB below 12 gm per dl and HCT below 36 percent were considered to be anemic (13,14). The anemia was defined as microcytic if the MCV was less than 70 femtoliters (fl) for infants and children younger than 2 years, less than 73 fl for children from 3 to 4 years, and less than 75 fl for children 5 years and older (15). Children with normal CBCs received no additional tests; children with abnormal CBCs were studied further. Additional tests performed on children who were identified as having a microcytic anemia included serum iron (SI), serum transferrin (ST), transferrin saturation (TS), and serum ferritin (SF). They were also given a four-week trial of iron therapy.

Table 1. Iron status of normal black children, ages 1 to 17 years, compared with that of children with iron overload, iron deficiency anemia, and thalassemia trait¹

Status	Number of children	SI ($\mu\text{g per dl}$)	ST ($\mu\text{g per dl}$)	TS percent	SF (ng per ml)	HI ($\mu\text{g per gm} \pm \text{SE}$)
Normal	20	ND	ND	ND	ND	61.9 \pm 10.3
Iron overload	3	93	218	49.7	593.3	34.0 \pm 9.2
Iron deficiency anemia	7	22	337	7.4	19.8	50.0 \pm 11.9
Thalassemia trait	6	74	328	22.7	47.4	94.5 \pm 26.7

¹ Mean values were obtained by clinical laboratory methods except for hair iron, which was obtained by the energy dispersive X-ray fluorescence (EDXRF) method.

NOTE: SI=serum iron; ST=serum transferrin; TS=transferrin saturation;

SF=serum ferritin; HI=hair iron; $\mu\text{g per dl}$ =micrograms per deciliter; ng per ml=nanograms per milliliter; $\mu\text{g per gm} \pm \text{SE}$ =micrograms per gram plus or minus standard error of the mean; ND=not done.

Iron overload. Patients were diagnosed as having iron overload if they had a TS of more than 62 percent or an SF of more than 300 nanograms per milliliter (ng per ml) or both for males and more than 200 ng per ml for females (16). Three previously diagnosed children were recruited from the pediatric hematology service for this portion of the study.

Iron deficiency anemia. Anemic children who fulfilled two or more of the following criteria: SI less than 25 $\mu\text{g per dl}$, ST more than 350 $\mu\text{g per dl}$, TS less than 17 percent, SF less than 20 ng per ml, and FEP 30–150 $\mu\text{g per dl}$ received a presumptive diagnosis of iron deficiency anemia (17,18). The diagnosis of iron deficiency anemia was confirmed by a rise of the HB and HCT to normal levels in response to iron therapy. Because clinical serum iron studies were not performed on children who had normal CBCs, children who were iron deficient but not anemic were not identified (19).

Thalassemia trait. Thalassemia trait was a diagnosis of exclusion in children with microcytic anemia who had normal SI, ST, TS, SF, and normal FEP levels. The diagnosis of thalassemia trait was confirmed by failure of the MCV to rise to a normal level after the child was maintained on therapeutic doses of ferrous sulfate for 4 weeks. Three children had "compensated anemia," with normal HB and HCT values, but all six had low MCV and high RBC values. The presence of a hemoglobin A₂ level of more than 3.5 percent established the diagnosis of beta rather than alpha thalassemia trait; however, the thalassemias were not differentiated for the purpose of this analysis.

Results

Of the 51 children who were examined, 48 were evaluable for the study. Three had iron overload, 7 had iron deficiency anemia, 6 had thalassemia trait,

20 were normal children ages 1 to 17 years, and 12 were normal infants younger than 1 year. The three children with iron overload had moderately severe hemolytic anemias; two had homozygous sickle cell disease (SS), and the third had hemoglobin H disease (a three-deletion alpha thalassemia syndrome). The three children excluded from the analysis included one who had concurrent iron deficiency and thalassemia trait, one with transient anemia of acute inflammation associated with a preceding infection that was resolved without specific treatment, and one with a mild normocytic anemia for which no etiology was found that appeared to be physiologic.

The 48 children ranged in age from 5 weeks to 17 years. There were 17 boys and 31 girls. All of the children were black. Heights and weights were within normal limits for age. All except 1 of the 12 infants had been born at full term with normal birth weights. The single exception, an infant born at 37 weeks gestation with a birth weight of 2,155 grams, was at the 75th percentile for weight and the 50th percentile for length when sampled at 4 months of age. None had acute infections, neurological deficits, or developmental delays. Peripheral blood values were available for only two of the infants. All of the children had normal blood lead levels, as determined by the District of Columbia screening laboratory. Low G6PD values and AS hemoglobin electrophoresis results were not considered reasons for exclusion from the study. The children resided in the Metropolitan Washington area, with zip codes 20001 through 20023, 20706, 20783, and 20785.

Hair treatment. Standard commercial shampoos, mostly "baby" shampoos, were used for most of the children. One girl's hair had been permanently waved and another girl's had been straightened. Because both had hair mineral values within the range for the other normal children, their results were included in the study.

Table 2. Distribution of minerals in hair of 12 normal black infants, ages 5 weeks through 12 months

Mineral	Median ²	Mean ²	Standard error
Calcium	672.4	698.9	68.6
Chromium	3.31	2.87	0.49
Manganese	2.78	2.84	0.32
Iron	30.1	38.1	6.8
Nickel	1.82	2.16	0.35
Copper	22.1	24.2	2.0
Zinc	128.8	123.8	20.9
Selenium	0.85	1.03	0.15
Bromine	12.3	15.7	2.9
Rubidium	3.43	4.00	0.69
Strontium	2.04	2.49	0.35
Lead	16.16	16.6	3.0
Mercury	1.03	1.15	0.33
Sulfur	50.6	58.0	7.0
Arsenic	1.33	1.68	0.34

¹ Only 10 were tested for sulfur.

² Mineral values are expressed in µg per gm except for sulfur, which is expressed in mg per gm.

A normal child, whose hair had been treated with vaseline, had excessively high lead and arsenic concentrations, but his blood lead and FEP levels were normal. A child with thalassemia trait, who had been shampooed with a special shampoo (Selsun Blue), had a selenium concentration 200 times that of the other subjects in the study. The aberrant hair lead, arsenic, and selenium values were considered to be caused by contamination and were excluded from the analysis.

Clinical laboratory findings. The normal children had a mean HB value of 12.1 gm per dl. Because only half of the thalassemic children were anemic, their mean HB value was 11.2 gm per dl. The iron overloaded children were anemic with a mean HB value of 7.7 and the iron deficient children were anemic with 9.8 gm per dl. The mean MCV values for children with iron deficiency anemia (69 fl) and thalassemia trait (67 fl) were low and almost identical compared with the value for normal children (80 fl). The mean RBC value was much higher for children with thalassemia trait [5.1 million per cubic millimeter (mil per cu mm)] than for children with iron deficiency anemia (4.4 mil per cu mm).

As shown in table 1, the mean TS level for children with iron overload was high (49.7 percent) compared with children with iron deficiency anemia (7.4 percent), while the level for children with thalassemia trait (22.7 percent) fell between those values. The mean SF level for children with iron overload was extremely high (593.3 ng per ml) compared with children with iron deficiency anemia

(19.8 ng per ml). The mean SF level for children with thalassemia trait (47 ng per ml) was above the level for children with iron deficiency anemia but within normal limits.

Minerals. Mean values for concentrations of 15 minerals in hair of 12 normal infants and 20 normal children are listed in tables 2 and 3.

Mean serum mineral and standard error values for 11 normal children were as follows:

Mineral	Mean value (µg per dl)	Standard error (µg per dl)
Iron	117.5	± 13.2
Copper	141.0	± 13.4
Zinc	160.1	± 14.6
Selenium	13.3	± 0.5
Bromine	525.5	± 55.7
Arsenic	1.83	± 0.28

Mean urine mineral and standard error values for 3 normal children were as follows:

Mineral	Mean value (µg per dL)	Standard error (µg per dL)
Manganese	31.3	± 4.3
Iron	64.0	± 5.0
Copper	257.3	± 55.4
Zinc	717.3	± 432.9
Selenium	30.7	± 0.7
Bromine	4,227.3	± 489.7
Lead	17.3	± 5.9
Mercury	6.50	± 2.18
Arsenic	8.33	± 1.89

Mean values for concentrations of 15 minerals in hair and serum of normal children and children with iron overload, iron deficiency anemia, and thalassemia trait are listed in table 4.

Mean serum mineral and standard error values for 4 children with thalassemia trait were as follows:

Mineral	Mean value (µg per dl)	Standard error (µg per dl)
Iron	118.6	± 12.6
Copper	167.8	± 16.2
Zinc	221.2	± 16.0
Selenium	13.3	± 0.9
Bromine	594.0	± 103.6
Arsenic	1.95	± 0.52

Hair iron. Mean hair iron concentrations for all of the children in the study are compared in table 4. When the individual hair iron values for each child are plotted (see chart), the mean values for children with iron deficiency anemia and normal children are similar. The mean value for the iron overloaded children, unexpectedly, is lower than the mean values for both normal children and children with

iron deficiency anemia. The mean value for the children with thalassemia trait is higher than mean values for the other three groups.

Statistical analysis. Median, mean, and standard error of the mean were calculated for each of the mineral values. Hair mineral values for the four groups of children were compared. A one-way statistical analysis of variance with no repeated measures, followed by a Scheffe post-hoc test to test the differences, revealed no significant differences among any of the four groups. Serum mineral values for the children with thalassemia trait were similarly compared with those of the normal children. There were no significant differences between the two groups.

Discussion

Iron deficiency is the most common nutritional deficiency and the most common cause of anemia in children. Even mild iron deficiency, with or without anemia, can cause developmental delays and behavioral deficits in the young (20-23). There is increasing awareness that iron overload, although uncommon, can also occur in children. It usually results from chronic transfusion programs or hemolytic anemias, but it also may be associated with the hereditary hemochromatosis gene (16). Because these conditions are treatable and because their side effects can be serious, a simple screening test such as hair iron analysis, if dependable, could represent an important advance in pediatric health care.

There was no significant difference between the concentrations of iron from the hair of our study children with iron overload and those with iron deficiency anemia, nor did the hair iron concentrations obtained from either group differ from those obtained from the normal children or the children with thalassemia trait. The iron overloaded group was clearly separated from the iron deficient group, with markedly elevated serum ferritin levels indicating large body iron stores in contrast to the depleted iron stores of the iron deficiency anemia group. Surprisingly, the mean hair iron concentrations seemed to suggest an inverse relationship, with the mean value for iron overloaded children being substantially lower than the mean concentration for iron deficient children. The amounts of storage iron in the two groups were so strikingly different that a significant difference should have been evident. In spite of the widely divergent SF levels between the two groups, statistical analysis revealed no significant difference in mean hair iron

Hair iron concentrations in 3 black children with iron overload, in 7 with iron deficiency anemia, and in 6 with thalassemia trait compared with those in 20 normal black children

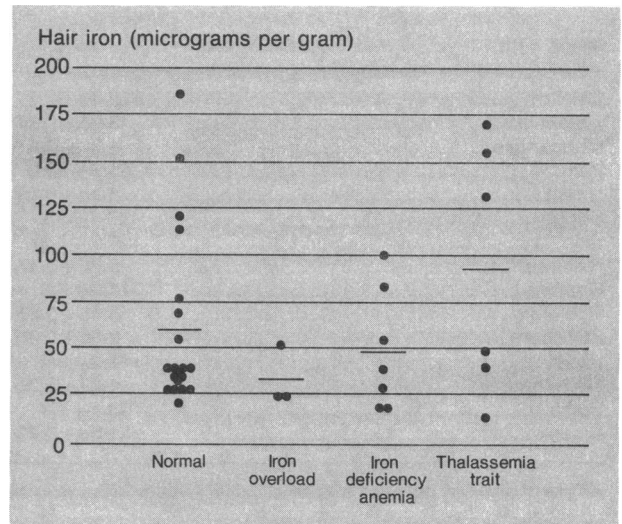


Table 3. Distribution of minerals in hair of 20 normal black children, ages 1-17 years

Mineral	Median ²	Mean ²	Standard error
Calcium	469.5	512.4	52.5
Chromium	2.34	3.06	0.54
Manganese	2.15	2.64	0.42
Iron	40.6	61.9	10.3
Nickel	1.67	3.18	0.93
Copper	23.0	27.4	3.2
Zinc	133.4	127.2	7.9
Selenium	0.72	0.82	0.13
Bromine	10.5	18.0	3.8
Rubidium	2.71	3.15	0.55
Strontium	1.68	2.32	0.36
Lead	9.5	12.3	2.4
Mercury	0.99	1.35	0.26
Sulfur	42.4	53.5	7.1
Arsenic	0.64	1.00	0.20

¹ One value each was deleted from the lead and arsenic analyses because of contamination.

² Mineral values are expressed in µg per gm except for sulfur, which is expressed in mg per gm.

levels. Hair iron analysis does not appear to be a reliable method for evaluating iron status in children, although our sample population is too small to make definitive statements.

In thalassemia trait, the inability to use iron in the incorporation of iron into functional porphyrins may be reflected by the inorganic iron being incorporated into hair (or excreted into urine), as also occurs with zinc and copper in some situations. On the other hand, there does not seem to be a ready explanation for the low mean iron concentration in the hair of the iron overload group and

Table 4. Mean mineral values¹ in hair of 20 normal black children, ages 1 to 17 years, compared with mean values in hair of 3 black children with iron overload, 7 with iron deficiency anemia, and 6 with thalassemia trait

Mineral	Normal values	Iron overload	Iron deficiency anemia	Thalassemia trait
Calcium	512.4 ± 52.5	383.8 ± 99.9	491.2 ± 78.8	456.8 ± 65.7
Chromium	3.06 ± 0.54	1.53 ± 0.53	1.90 ± 0.15	6.32 ± 2.71
Manganese	2.64 ± 0.42	1.58 ± 0.16	1.5 ± 0.028	2.93 ± 0.79
Iron	61.9 ± 10.3	34.0 ± 9.2	50.0 ± 11.9	94.5 ± 26.7
Nickel	3.18 ± 0.93	2.84 ± 1.10	3.35 ± 0.63	4.23 ± 1.67
Copper	27.4 ± 3.2	18.5 ± 5.6	42.3 ± 12.3	30.3 ± 4.0
Zinc	127.2 ± 7.9	118.7 ± 23.8	144.0 ± 22.1	144.0 ± 31.3
Selenium	0.82 ± 0.13	0.57 ± 0.13	0.96 ± 0.17	0.74 ± 0.10
Bromine	18.0 ± 3.8	19.9 ± 4.2	14.2 ± 3.0	21.8 ± 8.0
Rubidium	3.15 ± 0.55	3.46 ± 0.90	3.44 ± 1.24	3.95 ± 1.08
Strontium	2.32 ± 0.36	2.46 ± 1.43	4.26 ± 2.16	1.47 ± 0.23
Lead	12.3 ± 2.4	15.1 ± 6.8	14.1 ± 4.3	17.6 ± 6.7
Mercury	1.35 ± 0.26	0.64 ± 0.30	0.62 ± 0.12	1.39 ± 0.57
Sulfur	53.5 ± 7.1	36.9 ± 1.7	40.2 ± 8.1	39.2 ± 2.4
Arsenic	1.00 ± 0.20	1.34 ± 0.33	0.91 ± 0.23	1.07 ± 0.31

¹ Mineral values are expressed in µg per gm ± SE except for sulfur, which is expressed in mg per gm ± SE.

the high mean iron concentration in the hair of the iron deficiency anemia group, as compared with each other and with the normal children. One consideration is that there was overlap between the individual iron concentrations obtained from iron overloaded children and children with iron deficiency anemia. Another consideration is that the normal group of children may have included some who had iron deficiency without anemia (19). Neither of these factors appears to offer a satisfactory explanation, however, for the unexpected mean iron concentrations in the hair of children with abnormal iron metabolism. Further investigation with larger numbers of subjects would appear to be warranted.

Hair zinc values obtained from the normal black children in our study (table 3) did not differ appreciably from values obtained from normal children in Panama, Turkey, Brazil, Germany, Canada, Africa, and elsewhere in the United States (10,24-28). Likewise, hair zinc values obtained from the normal infants in our study (table 2) did not differ appreciably from values obtained from normal American infants by Collip and colleagues (29,30). Our values were lower, however, than values obtained from normal Brazilian infants by Dorea and Paine (26).

Hair copper values from our group of normal black children were similar to those of normal Panamanian and American children in other studies (10,31). Hair copper values from our group of normal black infants were similar to those of normal Canadian infants studied by Friel and colleagues (30).

Bromine levels in hair, serum, and urine of the normal black children in our study were consistent with levels reported in adult males by Cuenca and colleagues (32). Our subjects' hair bromine levels were higher than levels reported in normal children by Sky-Peck (10). Our manganese values, however, were similar to values found by Sky-Peck. Hair manganese values from our normal black infants were ten times higher than values found in normal Canadian infants by Friel and colleagues (30).

Hair mineral concentrations that were essentially the same for normal black children in our study and children in Sky-Peck's study included calcium, manganese, copper, zinc, selenium, rubidium, strontium, and sulfur. Our children had higher concentrations of iron, nickel, bromine, rubidium, lead, mercury, and arsenic. The differences are largely unexplained, although geographic location may have been a factor. Our subjects lived in metropolitan Washington, DC, while most of Sky-Peck's subjects lived in or near Chicago. Also, there were fewer patients in our study, and it is possible that with larger numbers the differences would have been less pronounced. Sky-Peck found racial differences, with blacks having significantly higher hair calcium, chromium, manganese, iron, nickel, lead, and arsenic concentrations than whites, but significantly lower hair rubidium, mercury, and sulfur concentrations. The racial differences are unexplained, although, again, geographic location should be taken into account. Various exposures, with industry, automobile exhaust fumes, lead plumbing, lead based paint, and well water influencing mineral absorption, may have played a role.

Hair, serum, and urine mineral concentrations from normal black infants and children, as we report, can serve as reference values. Mineral concentrations obtained from the hair and serum of children with iron overload, iron deficiency anemia, and thalassemia trait do not appear to be useful for defining these clinical conditions, although a larger sample population may have yielded more definitive results. Hair iron concentrations, in particular, do not appear to reliably reflect body iron deficiency or iron overload.

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Equipment

A. Kevex, 1101 Chess Drive, Foster City, CA 94404.