Human nails and body iron

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SUMMARY The iron content of human nails has been measured by atomic absorption spectrophotometry and compared with other measurements of iron status including the bone marrow. Four groups of individuals were studied: 40 healthy laboratory staff, five iron-deficient subjects before and during iron therapy, four patients at various stages of treatment with iron, and 15 postmortem cases. The iron status of the individual was reflected by the amount of iron present in nail samples. Nail sampling is proposed as a cheap, noninvasive method of assessing the iron status of the individual.

The iron in a sample of bone marrow forms the basis of the best available method of assessing tissue iron. The amount of iron in finger-nail clippings has been measured as an alternative assessment of tissue iron.

Patients and sampling

Samples of finger or toe nail clippings were obtained using stainless steel scissors or stainless steel nail clippers. It is possible to obtain satisfactory nail clippings every two weeks from the same nails. The minimum quantity of nail required for a satisfactory test is 20 mg.

Four groups of individuals were studied. In group 1 there were 23 female and 17 male healthy laboratory staff. Their ages ranged from 18 to 56 years. The findings expected in this laboratory in this group are as follows: haemoglobin concentration 12.3-16.5 g/dl for females and 13-18 g/dl for males, mean corpuscular volume (MCV) 80-96 fl, serum iron 13-24 $\mu\text{mol/l}$ (73-134 $\mu\text{g}/100\text{ ml}$), total iron binding capacity 45-80 $\mu\text{mol/l}$ (250-447 $\mu\text{g}/100\text{ ml}$), and a normal blood film appearance. In group 2 there were five iron-deficient subjects with a haemoglobin concentration of less than 12.3 g/dl, MCV less than 80 fl, serum iron less than 13 $\mu\text{mol/l}$, transferrin saturation less than 15%, with a total iron binding capacity greater than 45 $\mu\text{mol/l}$ (250 $\mu\text{g}/100\text{ ml}$), and hypochromic and microcytic blood films. The four bone marrow aspirates examined showed reduced or absent stainable iron using Perl's Prussian blue reaction. Group 3 consisted of four treated iron-deficient patients where

the diagnosis of iron deficiency had previously been made using the same criteria as in group 2. Group 4 consisted of 15 patients from whom nails and bone marrow had been obtained as part of a post-mortem examination.

Material and analysis

Liquid soap—an Izal product, IZAL Ltd. Supplied by Thorncliffe, Chapeltown, Sheffield, England.

Liquid soap solution—1 volume of liquid soap to approximately 15 volumes of glass distilled water.

4-Methylpentan-2-one (iso-butyl methyl ketone) for atomic absorption spectrophotometry. BDH Chemicals Ltd, Poole, England.

Nitric acid AR (1.42 sp. gr.). Supplied by Fisons Scientific Apparatus, Loughborough, England.

Iron solution 1000 ppm w/v of Fe in 0.1 N HClO_4 . Supplied by Hopkin and Williams Ltd, Chadwell Heath, Romford, Essex, England.

The iron was estimated using a flame technique and a 251 Atomic Absorption/Atomic Emission Spectrophotometer supplied by Instrumentation Laboratory, England. The readings were taken at wavelength 248.3 nm using the dual beam mode with an iron hollow cathode lamp, lamp current 8 mA, PM voltage 530 V, slit width 160 μm . Burner height 10 mm, fuel pressure 5 standard cubic feet per hour (SCFH), oxidant pressure 76 SCFH, aspiration rate 5 ml/min, integration—1 second manual.

If nail varnish had been used the nails were placed into glass universal containers to which 10 ml of iso-butyl methyl ketone was added and left for 45 minutes; the nails were then rinsed three times in the same iso-butyl methyl ketone. The above step was omitted if nail varnish had not been

applied. The samples were then treated as below:

The nail clippings were placed in plastic containers in which they were soaked in liquid soap solution for at least two to three hours and then washed free of debris and soap with iron-free water. The specimens were dried in an oven at 60°C to constant weight. The final dry weight of each specimen was recorded, and the specimens were placed into clean plastic centrifuge tubes to which 2 ml of nitric acid was added. Subsequently, the tubes were placed in a boiling waterbath for a few minutes until the nails dissolved completely. The tubes were cooled down. Four iron standards were prepared in the same nitric acid from an intermediate stock solution of 10 µg/2 ml. The four iron standards contained 0.1 µg, 0.2 µg, 1 µg, and 2 µg of iron in 2 ml nitric acid. Blanks of the same nitric acid as in the stock solution were frequently incorporated between the test readings. The final reading of each test was corrected for any drift of the blank reading. The instrument scale allowed values to be calculated to the nearest 1 µg/g. The serum iron and total iron binding capacity were estimated using an adaptation of a photometric method suggested by the International Committee for Standardisation in Haematology (Instrumentation Laboratory Inc., 1972) as a reference method.

TESTING OF DECONTAMINATION OF NAILS USING NAILS FROM NECROPSY CASES

A large homogenous nail sample was contaminated with soil, blood, and used car engine oil and subsequently subjected to the soap decontamination process. The iron content of contaminated nails was grossly increased, and decontaminated parts of the same nail showed a similar value to that of the uncontaminated nail portion.

A similar experiment was performed with a nail, part of which was contaminated with a nail varnish that contained iron. The results showed that the nail decontamination process was efficient.

Results

The iron content of the nails ranged from 6 to 26 µg per gram of nail for the women and 6 to 23 µg/g for the men in group 1. In 15 individuals from this group the test was repeated, and the average difference was less than 1 µg/g and the largest difference was 10 µg/g (Table 1).

The iron content of toe nails and finger nails was measured in 13 cases of group 1, and the average difference between the two in the same individual was less than 1 µg/g.

Group 2 consisted of patients with iron-deficiency

Table 1 Iron content of finger nails in healthy laboratory workers: group 1

	No. of cases	Age range (years)	Haemoglobin range (g/dl)	MCV range (fl)	Serum iron range (µmol/l)	TIBC range (µmol/l)	Nail iron	
							Range (µg/g)	Mean (µg/g)
Males	17	21-49	12.6-16.1	82-95	12.6-29.8	37-76	6-23	12
Females	23	19-56	12.6-15.0	83-96	13.1-38.1	36-80	6-26	13

Table 2 Iron content in nails in iron-deficient subjects: group 2

(a) Before treatment with iron

Case	Hb (g/dl)	MCV (fl)	Serum iron (µmol/l)	TIBC (µmol/l)	Bone marrow iron content where applicable	Iron content of nails (µg/g)
1	11.2	76	10.9	74	No stainable iron	Less than 1
2	10.7	81	4.2	92.9	Not done	3
3	4.6	60	Less than 1	76	No stainable iron	Less than 1
4	3.8	67	1.5	68	No stainable iron	Less than 1
5	6.7	56	3.1	79	Trace only	2

(b) During treatment with iron

Case	Hb (g/dl)	MCV (fl)	Serial measurements over a period of five weeks of the iron content of nails (µg/g)			
1	13.5	81	15	17	34	
2	14.4	81	8	15	21	
3	8.5	82	8			
4	5.6	—	2	Died after five days' treatment.		
5	10.3	70	18	22		

anaemia (Table 2a). All these patients were women and had less than 4 $\mu\text{g/g}$ of iron in their nails. In cases 1, 3, and 4 there was virtually no measurable iron. In these three cases there was no stainable iron in the bone marrow smears.

All group 2 cases were treated with iron, and the iron content increased over a period of five weeks, during which the nails were sampled (Table 2b). The nails became less brittle with parallel improvement in the well-being of the patients and in their haematological parameters. In case 4 the patient was transfused with 2 units of red cells and given Jectofer, 100 mg intramuscularly daily. She died from heart failure on the fifth day of her stay in hospital. At necropsy there was a trace of stainable iron in the bone marrow and 2 $\mu\text{g/g}$ of iron in the nails.

In group 3, in four patients who had recently begun treatment with iron for iron deficiency, the nail iron content was between 7 and 15 $\mu\text{g/g}$ (Table 3). None of them had a nail iron content less than 4 $\mu\text{g/g}$.

Table 3 Nail iron content in random patients being treated with iron: group 3

Case	Hb (g/dl)	MCV (fl)	Nail iron content ($\mu\text{g/g}$)
1	10.4	61	8
2	10.5	79	16
3	11.0	78	9
4	9.2	86	7

The postmortem studies in group 4, comparing the iron content in nails with that in the bone marrow, are shown in Table 4. Ten subjects showed normal bone marrow iron and a nail iron content of 68-205 $\mu\text{g/g}$. Three subjects showed a reduction of both stainable marrow iron and nail iron, and one showed an absence of stainable bone marrow iron and less than 1 $\mu\text{g/g}$ of nail iron. Iron distribution in the nails was also studied in this group by measuring the amount of iron in the proximal and distal parts of the nails of the same individual (Table 4). The smallest difference is 0 $\mu\text{g/g}$ and the largest 4 $\mu\text{g/g}$. The average difference was 1 $\mu\text{g/g}$ of nail.

Discussion

For many years there have been attempts to find a suitable method of assessing the iron status of man. The bone marrow iron content has been considered the best clinical method of assessing the iron status of the individual (Dagg and Goldberg, 1973). However, it is a qualitative method only.

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Table 4 Postmortem study of iron content in nails and bone marrow: group 4

Case	Nail iron content ($\mu\text{g/g}$)			Bone marrow iron content
	Total	Proximal half	Distal half	
1	6	6	6	Normal
2	21	22	20	Normal
3	2	2	2	Trace only
4	7	8	6	Normal
5	7	7	7	Slightly
6	2	2	2	Trace only
7	10	11	9	Normal
8	11	13	9	Normal
9	11	11	12	Normal
10	9	10	8	Normal
11	<1	<1	<1	Absent
12	3	3	2	Trace only
13	7	7	8	Normal
14	19	—	—	Normal
15	18	—	—	Normal

There are also discrepancies in the results of the other quantitative methods measuring bone marrow iron content as measured by isotope dilution technique and response to repeated phlebotomies (Gale *et al.*, 1963), reflecting different aspects of stored iron. The iron content of a tissue known to be sensitive to depletion of iron, such as the nail, could give rise to estimations of early changes in total body iron.

Sweat has been found to contain 1.61 mg/l of iron, the majority of the iron being in the cellular component (Hussain and Patwardhan, 1959). The nails are formed from epidermal cells, of which the deeper layer constitutes the matrix of the nail, and the upper cells of that matrix differentiate into nail substance, which is hard keratin. The cells that differentiate into keratin are displaced towards the distal end over the epidermal cells of the nail bed, so the nail is an equivalent to the stratum corneum, to which multiple epithelial cells are firmly attached. The average rate of nail growth is 0.5 mm per week (Ham, 1965). Iron incorporated in nails probably derives from the epidermal cells, which are concerned in making the nail, and from the cells firmly attached to it. In all cases tested, the concentrations of iron in the proximal and distal parts were similar. This finding is in keeping with the above growth process.

Previous estimations of the iron content of nails have shown average concentrations in adults of 130-230 $\mu\text{g/g}$ (Jacobs and Jenkins, 1960) with higher values in younger persons under the age of 15. Goldblum *et al.* (1953) give values of iron content in fresh human nails of 18-65 $\mu\text{g/g}$. The present results are 10 to 20 times lower than those of Jacobs and Jenkins and one-third of those of Goldblum *et al.*

The methods of iron measurement in each series are different, and sampling details are incomplete.

We have found that it was necessary to use Analar reagents and stainless steel scissors or nail clippers. The use of non-stainless steel instruments produced, in our sampled material, falsely high iron content. Morgan and Walters (1963) have investigated the iron content of spleen and liver in postmortem cases. They found that average storage iron concentration in the liver of normal subjects was $244.5 \pm 32.1 \mu\text{g/g}$ of iron.

The present study shows a relationship between nail iron and iron deficiency anaemia. Successful treatment of the anaemia with clinical improvement is coincident with increases in nail iron. Whether the amount of iron in the nail reflects the total body iron or the mobilisable iron demands further investigation.

Addison *et al.* (1972) described an immuno-radiometric assay for serum ferritin. Serum ferritin has been shown by Walters *et al.* (1973) to be directly related to storage iron status. Serum ferritin is related to iron stores in patients with iron deficiency or iron overload in the absence of liver disease, malignancy, inflammation, or increased red cell turnover within effective erythropoiesis (Jacobs *et al.*, 1972; Lipschitz *et al.*, 1974).

It is known that the rate of release of ferritin from tissues and the rate of removal of ferritin from plasma affect the serum ferritin. Changes in reticuloendothelial iron are rapidly followed by changes in the concentration of serum ferritin (Jacobs, 1976). Although serum ferritin has not yet been shown to correlate with nail iron, this study shows a speedy redistribution of iron to the nails in patients treated with iron in group 2. If this is confirmed it is possible that nail iron measurements may prove to be a method of determining whether iron therapy is being taken.

In iron-depleted and iron-sufficient subjects there was a correspondence between iron content of the nails and bone marrow iron, serum iron and TIBC, and red cell appearance and clinical status. In iron deficiency other mainly epithelial changes occur with abnormal keratinisation of the epithelium. The oral mucosa in iron deficiency often appears atrophic with glossitis and angular stomatitis (Beveridge *et al.*, 1965). Waldenström and Hallén (1938) describes epithelial nail changes in most patients with iron depletion. The commonest change was brittleness. All the patients in group 2 complained of brittle nails. This improved with treatment and, in parallel with clinical and haematological improvement and well being, there was a steady increase in nail iron content.

The method described here is simple, and sam-

pling is not invasive and is well tolerated. The samples are best taken by the patient and can be sent by post. The analysis requires a high-performance atomic absorption instrument, such as is used for the estimation of lead in whole blood. The only technical difficulty encountered, leading to over-estimation, occurred when other than stainless steel clippers or scissors were used or varnished nails were sampled. The treatment described using isobutyl-methyl ketone allows measurement of iron in varnished nails.

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