Bone marrow and chelatable iron in patients with protein energy malnutrition

A. A. Sive, W. S. Dempster, S. Rosseau, M. Kelly, H. Malan, H. de V. Heese

Objectives. To examine the iron status of malnourished children by comparing bone marrow iron deposits in children with protein energy malnutrition with those in well-nourished controls, and measuring chelatable urinary iron excretion in children with kwashiorkor.

Design. Bone marrow iron was assessed histologically in postmortem specimens from children with kwashiorkor or marasmus, and from controls. Twenty-four-hour urinary iron was measured in children with severe kwashiorkor, half of whom received 10 mg/kg of intramuscular desferrioxamine (DFO) on admission.

Setting. Red Cross War Memorial Children's Hospital, Cape Town.

Subjects. Thirteen children with kwashiorkor, 6 with marasmus and 16 well-nourished children underwent bone marrow examination. Urinary iron excretion was assayed in 17 children with kwashiorkor.

Results. Stainable iron was present in the bone marrow of half the children with kwashiorkor but in only 1 child in each of the other groups. The median iron excretion was 945.5 µg/24 hours in the DFO group compared with 28.5 µg/24 hours in the non-DFO group.

Conclusions. There is an apparent excess of iron which may predispose to bacterial infections and free radical-mediated injury in children with kwashiorkor.

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Iron acts as a catalyst in Haber-Weiss and Fenton-type reactions, causing the production of noxious tissue-damaging hydroxyl-free radicals associated with diseased states.[1,2] Free radicals are thought to be important in the genesis of kwashiorkor. There is evidence that iron for the promotion of free radicals may be available in patients with

Departments of Paediatrics and Child Health and Pathology, Red Cross War Memorial Children's Hospital and University of Cape Town

A. A. Sive, M.D., F.C.P.

W. S. Dempster, F.I.M.L.S. (U.K.)

S. Rosseau, NAT. DIP. M.T.

M. Kelly, M.B. CH.B., M.MED. (PATH.)

H. Malan, R.N., B.CUR. (NURS.)

H. de V. Heese, B.SC., M.D., D.C.H., F.R.C.P. (EDIN.)

kwashiorkor.[3,4] An apparent excess of iron in the presence of diminished iron-binding proteins would promote bacterial overgrowth, increasing the risk of systemic infection.[5]

Plasma ferritin and non-protein-bound iron concentrations are increased in patients with kwashiorkor.[6] However, to date it has not been possible to establish whether this ferritin is iron-rich or an apoferritin devoid of iron. Even though an excess of stainable iron has been reported in the liver,[7] only scanty deposits were found in the bone marrow of children with kwashiorkor.

This paper produces further evidence that iron is available for promoting free radical production in patients with kwashiorkor. Studies were carried out to assess deposits of stainable iron in the bone marrow and to demonstrate that chelatable iron is present and is excreted in the urine of children with kwashiorkor.

Patients and methods

All the patients in these studies conformed to the Wellcome classification of protein energy malnutrition.[8] Autopsy specimens of bone marrow were prepared for examination of iron deposits in 13 children with kwashiorkor, 6 with marasmus and 16 well-nourished children who had died of meningitis. The age range of the normally nourished children was 5 - 36 months, of the marasmic children 3.5 - 11 months and of the kwashiorkor children 4 - 66 months. Prussian blue staining (Perl's acid-ferrocyanide reaction) was used to demonstrate iron in the marrow preparations, all of which were evaluated by one observer (M.K.).

Seventeen male children with kwashiorkor admitted consecutively on weekdays to Red Cross War Memorial Children's Hospital were included in the iron chelation part of the study. Only boys were studied to facilitate the collection of urine. All had growth failure, generalised oedema, a typical skin rash, discoloration of the hair and hypo-albuminaemia of less than 20 g/dl. On admission, 9 randomly selected children received 10 mg/kg of desferrioxamine (DFO) (Desferal; Ciba) by intramuscular injection (DFO group). In the patients with kwashiorkor a venous blood sample was drawn for routine biochemical and haematological measurements, as well as for ferritin and transferrin. Urine was collected in acid-washed, iron-free glass containers during the first 24 hours for the estimation of iron. All infants received crystalloid intravenous fluids and were fed a commercial soya formula during the period of the study. Iron and trace metal supplements were withheld but the children received additional potassium and anthelmintics. Antibiotics were given according to laboratory and clinical indications. Dietary iron was only supplemented after the oedema had resolved.

Chelated iron was measured by a simplified wet ashing technique and spectrophotometric estimation, according to the bathophenanthroline sulphonate method.[9]

Data were analysed using non-parametric statistics.[10] Discrete data were compared by means of Fisher's exact test; correlation was assessed with the Spearman rank correlation coefficient; differences in plasma and urine concentrations between the groups were compared with the Mann-Whitney U test. Significance was accepted at the 5% level.

Informed consent was obtained from the custodial parent of each child. The research protocol was approved by the Ethics and Research Committee of the University of Cape Town.

Results

Stainable iron was found in the bone marrow of half the children with kwashiorkor but in only 1 patient in each of the other two groups (Table I).

Table I. Bone marrow iron

	No.	Iron present	Iron absent
Control	16	1	15
Kwashiorkor	13	7	7*
Marasmus	6	1	5

* $P = 0.02$ v. control (Fisher's exact test).

Iron was present in the urine of all the children who had received DFO and in 6 of the children who had not. Twenty-four-hour urine collection was successful in 8 children in the DFO group and in 6 of the others. Table II shows the haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), transferrin, transferrin saturation, ferritin and urine iron concentration as well as the 24-hour urinary iron excretion. The median urinary iron concentration in the DFO group was 262.0 µg/dl, significantly greater ($P < 0.001$) than that of 2.27 µg/dl in the non-DFO group. Similarly, the median 24-hour urinary iron excretion was also significantly greater ($P < 0.005$) in the DFO group (945.5 µg v. 28.5 µg). No differences were found in blood or plasma measurements between the groups. The urinary excretion of iron did not correlate with plasma iron-binding protein concentrations in either the DFO group or the non-DFO-group.

Discussion

Significantly more patients with kwashiorkor had detectable iron in their bone marrow than did normally nourished children. Only 1 child in the marasmic group had detectable marrow iron deposition. These measurements were carried out on autopsy specimens and it is conceivable that technical problems may have influenced the findings. Despite this acknowledged shortcoming, it was not justifiable to use an invasive procedure to obtain bone marrow from our malnourished patients for non-clinical indications. In an attempt to address the problems of artefacts affecting the histological interpretation, samples which had undergone autolytic change were excluded from the analysis. Bone marrow biopsies taken for diagnostic purposes from well-nourished children were not used as controls. The latter samples were all obtained from children who had died of bacterial meningitis. It is possible that infection in the control subjects could have accounted for the differences observed between the groups. However, as septicaemia is a major cause of death in malnutrition and

Table II. Individual Hb, MCV, MCH, transferrin, transferrin saturation, ferritin, urinary iron concentrations and 24-hour urinary iron content in children with kwashiorkor

Patient No.	Hb (g/dl)	MCV (fl)	MCH	Transferrin (g/l)	Transferrin saturation (%)	Ferritin (µg/l)	Urinary iron (µg/dl)	Urinary iron (µg/24 h)
Non-DFO								
1	12.7	67	21	1.21	Ins	26	3.3	4.9
2	6.6	77	25	0.46	84	226	0	0
3	6.5	89	29	1.05	45	258	1.64	480
4	11.4	76	25	0.72	52	121	0	0
5	10.0	76	25	0.73	71	132	6.5	52
6	7.8	76	24	1.67	3.7	414	2.9	97
7	11.0	78	32	0.55	77	278	8.95	Ins
8	6.3	69	22	0.38	74	195	0.75	Ins
DFO								
9	10.4	76	26	0.39	144	257	450	2 361
10	11.3	82	27	Ins	100	403	326	2 425
11	9.9	72	23	Ins	Ins	67	137	1 285
12	7.7	76	23	0.52	42	511	99	265
13	8.5	76	25	0.35	73	490	512	1 050
14	9.8	84	25	0.39	48	410	262	841 (Died day 6)
15	8.5	89	28	0.82	68	223	561	Incomplete
16				0.46	54	219	254	672
17	8.6	78	24	0.44	67	170	101	213

Patients 9 - 17 received an intramuscular bolus of 10 mg/kg desferrioxamine.

significant differences in bone marrow iron deposits were also found between patients with marasmus and kwashiorkor, it seems unlikely that infection alone is responsible for the findings. Adams and Scragg measured total iron-binding capacity and graded bone marrow iron deposits in children with kwashiorkor.[11] Their study does not show a discernible relationship between bone marrow iron and plasma iron-binding capacity. We previously demonstrated that over 50% of children with kwashiorkor have free iron in their plasma.[4] We have not been able to ascertain the relationship between free iron and marrow iron, but it is our impression that patients with the highest quantities of free iron are those with the worst outcomes.

In iron-overloaded subjects, urine iron levels increase markedly after the administration of the potent iron chelator, DFO, and the level can be used as an index of iron storage.[9,12,13] In response to chelation, the kwashiorkor children excrete large amounts of iron.[14] Following DFO,[9] the urinary iron excretion in half of our children was more than the reported upper limit of 1 200 µg per day for normal adults. Even though this limit was not exceeded by all the children, it is interesting that much iron was lost, given that there is a propensity to develop iron-deficiency anaemia during recovery from kwashiorkor. None of the children who received DFO had any detectable adverse effects, either in the short or the long term. They received iron supplements in therapeutic doses after their oedema resolved and none developed iron deficiency anaemia later.

Two of the children in the non-DFO group had no detectable iron in their urine on the evidence of a bleomycin assay. Whether these children represent that group of kwashiorkor patients who do not have free iron on the basis of this test is unknown. However, it is not surprising that the non-DFO group showed only minimal iron excretion. Even in patients with haemochromatosis, little iron is found in the urine unless they are given DFO.[12] We are unable to demonstrate a relationship between the plasma ferritin concentration and the amount of iron excreted in 24 hours or the urine iron concentration in any of the groups. Failure to do so may be a consequence of the small number of children studied. It may also indicate that elevated plasma ferritin levels in this context are evidence of an acute phase protein and do not indicate increased total body iron stores. Iron absorption in children with kwashiorkor is not abnormal.[15] Ramdath and Golden[14] speculate that the degree of bacterial contamination of the immediate environment and practices in food preparation result in increased siderophore levels in feeds and increased iron absorption which causes iron overload.[14] However, our findings of extremely low plasma transferrin concentrations associated with DFO-dependent urinary iron excretion suggest that in kwashiorkor the conventional circulating iron storage mechanisms are overwhelmed and that this necessitates an alternative short-term repository for endogenous iron. We speculate that during the period of increasing undernutrition preceding the onset of overt kwashiorkor, iron is removed from the circulation as the plasma iron-binding capacity progressively falls. This iron is deposited in the liver, bone marrow and ferritin, notwithstanding a state of overall iron insufficiency.

This sequestration of iron in tissue and ferritin may represent an attempt to inhibit bacterial overgrowth and consequent systemic infection. Iron sequestration may also play a role in limiting the production of reactive oxygen species via the Haber-Weiss reaction. The presence of circulating chelatable iron in kwashiorkor suggests that despite the body's adaptive responses in malnutrition, iron remains available to enhance free radical production. Once recovery takes place, there is a rapid utilisation of the stored

iron, resulting in a fall in the plasma ferritin concentration and consequent iron-deficiency anaemia.

It is reasonable to speculate that the chelatable iron is responsible for catalysing radical production. It is also feasible that non-chelatable iron is involved in the process and that DFO will not modulate free radical production. Nevertheless, our results suggest that a placebo-controlled trial of iron chelation therapy is warranted, aimed at limiting free radical-mediated injury during the early management of kwashiorkor with subsequent iron replacement.

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REFERENCES

1. Gutteridge JMC, Halliwell B. Iron toxicity and oxygen radicals. In: Hershko C, ed. *Clinical Haematology. Iron Chelating Therapy.* London: Baillière Tindall, 1989: 195-256.
2. Kleinveld HA, Swaak AJG, Hack CE, Koster JF. Interactions between oxygen, free radicals and proteins. *Scand J Rheumatol* 1989; **18:** 341-352.
3. Golden MHN. The consequences of protein deficiency in man and its relationship to the features of kwashiorkor. In: Blaxter K, Waterlow JC, eds. *Nutritional Adaptation in Man.* London: Applied Science Publishers, 1985: 169-187.
4. Dempster WS, Sive AA, Rosseau S, Malan H, Heese HdeV. Misplaced iron in kwashiorkor. *Eur J Clin Nutr* 1995; **49:** 208-210.
5. McFarlane H, Reddy S, Adcock KJ, Adeshina H, Cooke AR, Akene J. Immunity, transferrin and survival in kwashiorkor. *BMJ* 1970; **4:** 268-270.
6. Golden H, Golden BE, Bennett FI. Relationship of trace element deficiencies to malnutrition. In: Chandra RK, ed. *Trace Elements in Nutrition of Children.* New York: Raven Press, 1985: 185-207.
7. Waterlow JC. *Fatty Liver Disease in Infants in the British West Indies.* London: HMSO, 1948.
8. Gurney JM. The young child: protein energy malnutrition. In: Jelliffe DB, Jelliffe EFP, eds. *Nutrition and Growth.* New York: Plenum Press, 1979: 185-216.
9. Barry M. Determination of chelated iron in the urine. *J Clin Pathol* 1968; **21:** 166-168.
10. Siegel S, Castellan NJ jun. *Nonparametric Statistics for the Behavioural Sciences.* 2nd ed. New York: McGraw-Hill, 1988.
11. Adams EB, Scragg JN. Iron in the anaemia of kwashiorkor. *Br J Haematol* 1965; **2:** 676-681.
12. Bonkovsky HL, Weber R, Aaron L. Four-hour measurement of urinary iron excretion after desferrioxamine treatment: a rapid, simple method of study of iron excretion. *Am J Gastroenterol* 1990; **85:** 554-557.
13. Caballero B, Solomon US, Batres R, Toran B. Homeostatic mechanisms in the utilisation of exogenous iron in children recovering from severe malnutrition. *J Pediatr Gastroenterol Nutr* 1985; **4:** 97-102.
14. Ramdath DD, Golden MHN. Non-haematological aspects of iron nutrition. *Nutr Res Rev* 1989; **2:** 29-49.
15. Lynch SR, Becker D, Seftel H, Bothwell TH, Stevens K, Metz J. Iron absorption in kwashiorkor. *Am J Clin Nutr* 1990; **23:** 792-797.

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