Iron Stores in Health and Disease

Bone-marrow Studies in 1134 Children in Punjab, India

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In iron deficiency, depletion of iron stores precedes alterations in serum iron concentration, iron-binding capacity, and morphological abnormalities of red cells (Simon and Finch, 1964; Beutler, Robson, and Buttenwieser, 1958). The use of a simple and practical clinical procedure, introduced by Rath and Finch in 1948 for the semiquantitative assessment of stainable marrow iron, has enabled the clinician to diagnose this 'pre-clinical' stage of iron deficiency.

The definitive diagnosis of iron deficiency in the developing countries is particularly important because iron deficiency occurs far more frequently than hitherto suspected, and because this deficiency is often not isolated but occurs in association with other facets of malnutrition, and with infections.

The present study of 1134 subjects was undertaken to find the incidence of iron depletion in health, and to assess the prevalence of iron deficiency in various disease entities, as a basis for exploring the factors leading to the development of hypsiderosis in different clinical conditions. In addition, the comparative value of the various diagnostic parameters commonly used for diagnosing iron deficiency in clinical practice has been critically assessed.

Material and Methods

The study included 1134 children from 10 days to 14 years of age; 954 were suffering from various clinical disorders, and 180 were apparently healthy normal subjects. These 'normal' children were derived from the paediatric Follow-up-Clinic, Child Guidance Clinic, and from those convalescing from minor medical or surgical illnesses; none had suffered from any serious illness during the preceding 3 months of this study, nor had received iron medication orally or parenterally during the past 6 months. All the 1134 children under study were admitted and comprised the following five groups.

Group I—Normal (180 cases). These 180 children had Hb levels defined as follows (Wolman, 1957):

(a) 9 g./100 ml. in the age-group 1–9 months; (b) > 10 g./100 ml. in age-group 9 months to 6 years; and (c) > 11 g./100 ml. in age-group 6–14 years.

Group II—Malnutrition (521 cases). (a) Iron deficiency, 160 cases; (b) marasmus, 130 cases; (c) marasmic kwashiorkor, 90 cases; (d) scurvy, 20 cases; and (e) megaloblastic anaemia, 121 cases.

Group III—Chronic systemic infections (210 cases).

Group IV—Chronic non-infective systemic illnesses (183 cases). (a) Rheumatic heart disease, 40 cases; (b) cyanotic congenital heart disease, 28 cases; (c) portal cirrhosis, 40 cases; (d) nephrotic syndrome, 62 cases; and (e) cretinism, 13 cases.

Group V—Other blood disorders (40 cases). (a) Thalassaemia, 22 cases; (b) malignancies (leukaemias), 18 cases.

After recording the history and the physical findings in each case, the routine laboratory tests included tuberculin testing, urine and stool examination, the latter particularly for helminthic/protozoal infestation, and occult blood. The selective radiological and biochemical investigations were undertaken as and when indicated for establishing the aetiological diagnosis. The haematological studies comprised Hb estimation (Sahli's method, the accuracy of the apparatus was checked repeatedly by the cyanmethaemoglobin method, using the photo-electric colorimeter); red cell count; haematocrit determination (Wintrobe's method, 1967); detailed survey of the Leishman's stained peripheral blood smear; calculation of red cell indices; serum iron (SI); iron-binding capacity (IBC) (Ramsay's method, 1954, 1958); unsaturated iron-binding capacity (UBIC); and percentage saturation iron-binding capacity (% sat. IBC).

Samples of marrow* in children under 2 years were

* Following an inquiry by the Editors on the ethical aspects of their investigations, the authors write: 'Parental permission for undertaking the various investigations, including the "healthy normal" group, was obtained in each case. The presence of other children who had already been investigated served to allay any parental fears. No complication was met with after marrow puncture in any of the 1134 children, thus indicating the safety of this procedure.' *Editor.*

aspirated from the tibia; in older children from the ilium, 2 cm. posterior and inferior to the anterior superior iliac spine. The patients were sedated with chlorpromazine (1.5 mg./kg.), and local anaesthesia only was used.

The bone-marrow morphology was studied for qualitative abnormalities and rough quantitative assessment of cellularity, and the relative proportion of the various cellular elements. The stainable iron in the bone-marrow smears as marrow haemosiderin (MH) was graded 0 to 3+ according to Beutler's (Beutler *et al.*, 1958) criteria, and the staining technique used was that of Dacie (Dacie and Lewis, 1963). The interpretation of MH was, however, modified so that iron deficiency was considered to be present if the MH content was 1+ or less.

Observations

The final diagnosis of iron deficiency in each case was based on the absence or inadequacy of stainable iron in the marrow (i.e. graded 1+ or less). Thus, 76% of the total 1134 children (56% of the 180 healthy ones and 81% of the 954 ill children) were iron deficient by this criterion.

The incidence of iron deficiency by the other parameters in the various clinical groups of 1134 children as compared with the inadequate MH content is shown in Table I. From this it is seen that there was a higher incidence of iron deficiency in the ill subjects compared to the healthy ones, the difference being reflected by all the parameters.

In Group I—amongst the 180 healthy children, 27% were aged less than 9 months, 36% were between 9 months and 6 years, and 36% were over 6 years. Though the Hb levels of all these cases lay within the normal ranges for their respective age-groups, 23% were judged on film appearances to be microcytic, and 12% showed hypochromia. The incidence of iron deficiency derived from other parameters (see Table IV) was 56% with inadequate MH, 22% with low SI, 29% with low percentage sat. IBC, and 36% with low MCHC.

Table II gives the incidence of stainable iron, graded quantitatively, in marrow aspirates in the different groups. Table III gives Hb values in 954 ill children with various clinical disorders.

Table IV shows the incidence of iron deficiency by different diagnostic parameters in 1134 children. In the 521 malnourished children of Group II, the high incidence of iron deficiency (85%) as determined by MH differed little from the incidence inferred from examination of the blood film and MCHC, 77% and 73% respectively, whereas SI and percentage sat. IBC were indicative of iron deficiency in only 53% and 58% respectively.

Of the 160 children shown in Table IV, Group II (a) with nutritional iron deficiency anaemia, diagnosed on the basis of inadequate MH content, only 80% had hypochromic red cells, 88% low MCHC, 86% low SI, and 79% low % sat. IBC. Thus, without marrow investigation, 12 to 21% of cases of iron deficiency were undetected by routine blood examinations, including serum iron measurements.

In the 121 cases with megaloblastic anaemia (Table IV, Group II (b)) the incidence of inadequate MH was 79%, low percentage sat. IBC was 15%, low SI was 23%, low MCHC was 49%, though 73% had hypochromic red cells. Thus the chances of missing iron deficiency in megaloblastic erythropoiesis during early infancy are minimized.

In the 210 cases with chronic infections (Table IV, Group III) 56% of which had Hb < 8 g./100 ml., 73% showed hypochromic red cells associated with microcytosis in 43%, normocytosis in 17%, and macrocytosis in 12%. The incidence of iron deficiency in terms of MH was 83%.

Discussion

The high incidence of iron deficiency, 77% of the total 1134 children including 56% of the 180 healthy children, emphasizes the magnitude of iron deficiency in our child population. More than

TABLE I

Percentage of Cases Showing Iron Deficiency According to the Criteria of Marrow Haemosiderin, Peripheral Blood Film Appearances, MCHC, Saturation Index, and Percentage Saturation of Iron-binding Capacity*

	Marrow Haemosiderin	Blood Film	MCHC	SI	Sat. IBC
180 Healthy children	56	22	36	22	30
954 Ill children	81	82	70	51	52
1134 All children	77	73	65	86	52

* Iron deficiency was considered to be present if marrow haemosiderin was 0 or 1+; blood film showed hypochromic red cells; MCHC < 32% serum iron < 50 µg./100 ml.; saturation of IBC < 20%.

TABLE II
Grading of Marrow Haemosiderin in Different Clinical Groups

Clinical Group	Marrow Haemosiderin								Total
	0 or $\pm$		1 +		2 +		3 + or more		
	No.	%	No.	%	No.	%	No.	%	
I: Healthy children	18	10	82	46	80	44	—	—	180
II: Malnutrition	245	47	198	38	46	9	32	6	521
(a) Iron deficiency	140	87	20	13	—	—	—	—	160
(b) Marasmus	81	63	32	25	14	11	3	2	130
(c) Marasmic kwashiorkor	60	67	20	22	8	9	2	2	90
(d) Scurvy	14	70	—	—	4	20	2	10	20
(e) Megaloblastic anaemia	—	—	96	79	16	13	9	7	121
III: Chronic infections	116	55	58	28	30	14	6	3	210
IV: Chronic systemic illnesses	108	59	41	22	27	15	7	4	183
(a) Rheumatic heart disease	34	85	—	—	6	15	—	—	40
(b) Cyanotic congenital heart disease	16	57	4	14	8	29	—	—	28
(c) Cirrhosis	18	45	14	35	4	10	4	10	40
(d) Nephrosis	32	52	20	32	8	13	2	3	62
(e) Cretinism	8	62	3	23	1	8	1	8	13
V: Miscellaneous	1	3	4	10	14	35	21	53	40
(a) Acute leukaemia	1	6	4	22	6	33	7	39	18
(b) Thalassaemia	—	—	—	—	8	36	14	64	22

TABLE III
Haemoglobin Levels in 954 'III' Children

Disease	Haemoglobin Levels						Total
	< 4 g./100 ml.		4-8 g./100 ml.		> 8 g./100 ml.		
	No.	%	No.	%	No.	%	
Nutritional iron deficiency	47	29	82	51	31	19	160
Marasmus	26	20	79	61	25	19	130
Marasmic kwashiorkor	21	23	52	58	17	19	90
Megaloblastic anaemia	31	26	53	44	36	30	120
Scurvy	7	35	9	45	14	20	30
Chronic infective diseases	44	21	74	35	92	44	210
Rheumatic heart disease	—	—	11	28	29	73	40
Cyanotic congenital heart disease ..	—	—	10	29	24	71	34
Cirrhosis	12	21	23	41	21	38	56
Nephrotic syndrome	4	7	26	42	32	52	62
Cretinism	1	8	11	85	1	8	13
Thalassaemia	4	18	13	59	5	23	22
Leukaemia	4	22	14	78	—	—	18

half the children in the apparently well-nourished and non-anaemic healthy group showed depleted iron stores, despite haemoglobin levels and red cell indices within the normal range, indicating a high incidence of 'pre-clinical', 'masked', or 'latent' iron deficiency. Sturgeon (1956) and Undritz (1964) made similar observations. Normal red cell indices and serum iron values fail to indicate absent or marginal iron stores. Whether amounts of storage iron, subnormal in terms of adult standards, are to be regarded as 'physiological' for the growing child, has long been debated. We

consider that such marginal iron reserves are suboptimal, for owing to growth, reserves will be more quickly exhausted in the child than in the adult if there is inadequate intake, impaired absorption, or abnormal loss of iron.

If it is accepted that the most satisfactory criterion for iron deficiency is the finding of low amounts of haemosiderin in the marrow, this study has shown that the next most dependable diagnostic criterion is the presence of hypochromia of the red cells in peripheral blood films, whereas red cell indices, SI, and percentage sat. IBC are

TABLE IV
Incidence of Iron Deficiency* by Different Diagnostic Parameters

Total	Blood Film		MCHC		SI		Sat. IBC		Marrow Haemosiderin		
	No.	%	No.	%	No.	%	No.	%	No.	%	
I: Normal healthy	180	40	22	65	36	40	22	53	29	100	56
II: Malnutrition	521	402	77	379	73	277	53	300	58	445	85
(a) Iron deficiency anaemia	160	128	80	140	88	138	86	127	79	160	100
(b) Megaloblastic anaemia	121	88	73	60	49	27	23	18	15	93	79
(c) Marasmus	130	102	79	98	75	79	61	58	45	86	67
(d) Marasmic kwashiorkor	90	68	76	69	77	42	47	42	47	80	89
(e) Scurvy	20	16	80	12	60	11	55	5	25	14	70
III: Chronic infections	210	192	91	150	71	115	55	138	66	174	83
IV: Chronic systemic illnesses	183	155	85	111	61	95	52	84	46	149	81
(a) Rheumatic heart disease	40	36	90	16	40	23	58	17	43	34	85
(b) Cyanotic congenital disease	28	24	86	21	75	10	36	13	46	20	71
(c) Nephrotics	62	54	87	40	65	44	71	36	58	52	84
(d) Cirrhosis	40	31	78	28	70	13	33	15	38	32	80
(e) Cretinism	13	10	77	6	36	5	3	3	23	11	85
V: Other blood disorders	40	37	93	30	75	—	—	9	23	5	13
(a) Leukaemias	18	16	39	10	56	—	—	2	11	5	28
(b) Thalassaemia	22	21	96	20	91	—	—	7	32	—	—
Total	1134	826	73	735	65	527	47	584	52	873	77
Total 'ill' group	954	786	62	670	70	487	51	531	56	773	81

* For criteria for diagnosis of iron deficiency, see Table I.

less reliable for diagnosing iron deficiency. Cases of thalassaemia, leukaemia, and also cretinism, however, are exceptions to this generalization. We have also noted, as have others (Beutler *et al.*, 1958), the further exception that malnutrition and infections can themselves cause hypochromic red cells, as well as alter the serum iron values, in the absence of iron deficiency as defined by marrow haemosiderin.

Protein-calorie malnutrition and anaemia have a complex interrelation which no doubt varies with the nutritional background of the different regions of the world. In the present study, iron deficiency was present in 89% cases of protein-calorie malnutrition and in 66% of those with severe calorie insufficiency. This high incidence of iron deficiency, particularly in protein-calorie malnutrition, fits in broadly with the studies reported from other tropical countries (Brown, 1965).

The high incidence (83%) of iron deficiency anaemia in chronic infections needs comment. Chronic infections of a few weeks' duration tend to lead to normocytic-normochromic or normocytic-hypochromic anaemia, but not commonly to microcytosis. The degree of anaemia is usually not severe except in cases of certain severe infections, as for example, bacterial endocarditis, miliary tuberculosis (Corr, Kyle, and Bowie, 1964), or pulmonary suppuration (Wintrobe, 1967). The anaemia of infection is classically stated to be 'non iron-

deficiency', despite hypoferraemia (Heilmeyer, 1964). With these probable multifactorial pathogenetic factors present in children presenting with both anaemia and infection, assessment of iron stores by MH estimation is particularly valuable. The high incidence of iron deficiency in the present series is no doubt partly due to pre-existing iron deficiency. The anaemia, in turn, may perpetuate or worsen the infections, so that in this part of India 'anaemia of infection' should be more appropriately termed 'anaemia with infections'.

In the third main group of systemic non-inflammatory diseases, the diagnosis of iron deficiency complicating rheumatic or cyanotic congenital heart disease has practical significance, because of the possibility of relief of symptoms if these are due in part to iron deficiency. The presence of iron deficiency in cases of cyanotic polycythaemia of congenital heart disease is often overlooked, though it was present in the majority (76%) of these cases in the present study.

Summary

Bone-marrow haemosiderin was assessed semi-quantitatively in a series of 1134 children of all ages: 180 children were healthy, and the remaining 954 were suffering from a variety of disorders, mainly chronic. Iron deficiency was diagnosed if iron stores, as reflected by marrow haemosiderin,

were low or absent. With this criterion, iron deficiency was present in 56% of the healthy children. Iron deficiency was present in 81% of all the ill children; in those with malnutrition, the incidence was 85%, and in those with chronic infections, 83%.

Other commonly used criteria of iron deficiency were evaluated at the same time, including red cell film appearances, red cell indices, serum iron level, and percentage saturation iron-binding capacity. Of these, the presence of hypochromia of red cells proved the most generally useful indicator of iron deficiency, while those based on serum iron levels often proved misleading.

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