Serum and red cell folates, and serum vitamin B₁₂ in protein calorie malnutrition

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Khalil, M., Tanios, A., Moghazy, M., Aref, M. K., Mahmoud, S., and el Lozy, M. (1973). *Archives of Disease in Childhood*, **48**, 366. **Serum and red cell folates, and serum vitamin B₁₂ in protein calorie malnutrition.** In 22 cases of kwashiorkor, 19 cases of marasmus, and 16 normal controls, red cell folate, serum folate, and serum vitamin B₁₂ were estimated, and the bone marrow and peripheral blood examined. Erythrocyte folate deficiency was shown in 9 cases of kwashiorkor and 7 cases of marasmus. Serum folate deficiency was present in 14 cases of kwashiorkor and 7 cases of marasmus. Megaloblastosis was found in 45% of cases of kwashiorkor and 37% of cases of marasmus. Megaloblastosis and macrocytosis correlated more with erythrocyte than with serum folate deficiency. Serum vitamin B₁₂ levels in children with kwashiorkor or marasmus did not differ from those of normal controls. The role of folate deficiency in the pathogenesis of megaloblastosis in protein calorie malnutrition was confirmed.

A hypochromic anaemia of iron deficiency is a salient finding in patients with protein calorie malnutrition (Khalil, Awwad, and Hafez, 1968). However, an associated megaloblastic bone marrow reaction producing a dimorphic type of anaemia is in many cases demonstrable (Dean, 1965; Khalil *et al.*, 1970). In very few instances has the anaemia been of the pure megaloblastic type (Pereira and Baker, 1966).

In an attempt to study the pathogenesis of megaloblastic anaemias in kwashiorkor, Sandstead *et al.* (1965) estimated serum vitamin B₁₂, but found no correlation between it and megaloblastosis. Halsted *et al.* (1969) studied the level of serum folate in a group of patients suffering from the disease and reported a decreased level together with an increase in the level of serum vitamin B₁₂. Recently, however, the diagnostic value of red cell folate level as a reliable index of folic acid deficiency has gained increasing acceptance (Kitay, 1969).

The present paper deals with a comparative study of serum and red cell folates and serum vitamin B₁₂ in infants suffering from protein calorie malnutrition. The study sets out to correlate the haematological findings in these patients with the folate and vitamin B₁₂ status as determined by assays on blood.

Material and methods

The study was carried out on 22 infants (12 males and 10 females) suffering from kwashiorkor and 19 (7 males and 12 females) suffering from marasmus, together with a matching normal control group of 16 apparently well-nourished infants (8 males and 8 females). The age of the infants ranged between 6 months and 2 years.

The differentiation between kwashiorkor and marasmus was based on the classification of Jelliffe and Dean (1959) and Jelliffe (1966). In kwashiorkor, oedema, growth failure, muscle wasting with retention of some subcutaneous fat, and psychomotor changes were considered as constant features. Other signs such as hair changes, skin lesions, and hepatomegaly were common but not constantly present in our cases. Growth retardation, muscle wasting, and variable degrees of subcutaneous fat loss were constantly present in marasmus. Cases of marasmic kwashiorkor in which body weight is marked in spite of the presence of oedema were, however, not included in this study. Gastrointestinal disorders were associated in many of the studied cases.

The following laboratory investigations were carried out. Complete blood picture; iliac crest myelogram; estimation of total serum proteins; estimation of serum folates using *Lactobacillus casei* method (Waters and Mollin, 1961); estimation of red cell folates using *L. casei* method (Hoffbrand, Newcombe, and Mollin, 1966); estimation of serum vitamin B₁₂ using *Lactobacillus leishmanii* method (Matthews, 1962).

TABLE I
Comparative study of cases of kwashiorkor, marasmus, and normal controls

	Controls (16 cases)		Kwashiorkor (22 cases)			Marasmus (19 cases)		
	Mean	SD	Mean	SD	P	Mean	SD	P
Serum proteins (g/100 ml)	6.4	0.5	4.8	0.6	HS	6.2	0.5	NS
Haemoglobin (g/100 ml)	12.4	0.6	7.4	1.6	HS	8.6	1.3	HS
MCV (μ^3)	84.6	4.7	107.3	26.5	HS	102.5	19.0	HS
MCHC (%)	32.3	0.8	22.5	4.6	HS	23.3	4.4	HS
Mean number of lobes (%)	2.7	0.2	2.9	0.4	NS	2.8	0.4	NS
Marrow count (1000/mm ³)	175.1	60.2	57.2	30.6	HS	86.8	62.9	HS
Erythroid cells (1000/mm ³)	40.4	18.2	22.7	11.0	HS	30.4	20.2	NS
Erythrocyte folates (ng/ml)	306.6	137.8	137.9	92.8	HS	167.1	108.0	HS
Serum folate (ng/ml)	9.3	4.3	3.6	2.2	HS	4.5	3.0	HS
Serum vitamin B ₁₂ (pg/ml)	393.4	472.0	494.0	211.3	NS	411.7	197.2	NS

NS, nonsignificant difference, $P > 0.05$; S, significant difference, $0.05 > P > 0.01$; HS, highly significant difference, $P < 0.01$.
Note: Tests of significance are in each case between the named group and the controls.

Results

The main results are given in Table I. Serum proteins were significantly diminished in kwashiorkor but not in marasmus, as compared to the controls. In the peripheral blood in both types of protein calorie malnutrition the haemoglobin level and the mean corpuscular haemoglobin concentration (MCHC) were lower than normal, while the mean corpuscular volume (MCV) was significantly increased. There was no evident difference in the degree of nuclear segmentation of neutrophils in the 3 groups. In the bone marrow both kwashiorkor and marasmus showed a significant decrease in the total count of myeloid and erythroid cells. In 45% of the cases of kwashiorkor and 37% of those of marasmus the bone marrow showed a megaloblastic reaction, which was not seen in any of the controls. Both

serum and erythrocyte folate levels were much lower in kwashiorkor and marasmus than in normal controls. No significant differences, however, were found in the level of serum vitamin B₁₂ between the 3 groups.

Table II shows the number of cases classified according to marrow reaction and the level of erythrocyte and serum folates in the two groups of malnourishment. In the case of erythrocyte folates the correlation with the marrow picture is very close, with a χ^2 value of 26.86 for the two groups combined. In the case of serum folates, the correlation, though still statistically highly significant, is much less close, with a χ^2 value of 7.41 for the two groups combined.

Table III shows the relation between serum and erythrocyte folate levels in the malnourished infants.

TABLE II
Relation between marrow reaction and erythrocyte and serum folate level

	Normal RBC folate (above 100 ng/ml)	Low RBC folate	Normal serum folate (above 3 ng/ml)	Low serum folate
Normoblastic	22 cases (11 K + 11 M)	2 cases (1 K + 1 M)	16 cases (7 K + 9 M)	8 cases (5 K + 3 M)
Megaloblastic	3 cases (2 K + 1 M)	14 cases (8 K + 6 M)	4 cases (1 K + 3 M)	13 cases (9 K + 4 M)

K, kwashiorkor; M, marasmus.

TABLE III
Relation between serum and erythrocyte folate levels

	Erythrocyte folate (below 100 ng/ml)	Erythrocyte folate (above 100 ng/ml)
Serum folate (< 3 ng/ml)	11 cases (7 K + 4 M)	11 cases (7 K + 4 M)
Serum folate (3–5 ng/ml)	5 cases (2 K + 3 M)	4 cases (2 K + 2 M)
Serum folate (> 5 ng/ml)	0 cases (0 K + 0 M)	10 cases (4 K + 6 M)

K, kwashiorkor; M, marasmus.

It is seen that low erythrocyte folates are invariably associated with low, or at least lowered, serum folate values, while normal erythrocyte folates may be associated with either low or normal values of serum folates.

Discussion

In the present work a simultaneous study of serum and red cell folate levels together with serum vitamin B₁₂ was carried out to evaluate the possible role of each in the development of megaloblastic erythropoiesis. The red cell folate level is considered to reflect more accurately the state of folic acid stores than does the level in the serum. Serum folic acid *per se* cannot be accurately diagnostic of folic acid deficiency, since low serum folates, in the presence of normal or high red cell folates, were reported in cases of iron deficiency or toxic infections (Hansen, 1966). As previously mentioned, iron deficiency was common among our cases of protein calorie malnutrition (Khalil *et al.*, 1968).

Red cell folates in normal controls in this study varied between 110 and 580 ng/ml, with a mean of 306.6 ng/ml. The mean values of red cell folates in kwashiorkor and marasmus were 137.9 and 167.1 ng/ml, respectively, denoting a significant reduction. However, definite red cell folate deficiency was diagnosed if the level was below 100 ng/ml, which agrees with most workers (Chanarin, 1969). Red cell folate deficiency was encountered in 9 out of 22 cases of kwashiorkor and 7 out of 10 cases of marasmus.

There was a significant decrease in serum folate in protein calorie malnutrition compared to normal controls. The mean serum folate levels in kwashiorkor, marasmus, and normal controls were 3.62, 4.5, and 9.3 ng/ml, respectively. Serum folate deficiency below 3 ng/ml was present in 14 out of 22 cases of kwashiorkor, and in 7 out of 19 cases of marasmus. Table III shows that while low erythrocyte folate levels were always associated with low serum folate levels, the opposite was not true, as low serum folate levels could be accompanied by either normal or low erythrocyte folate levels. This may be explained on the basis of the experimental work done by Herbert (1962) in which serum folate deficiency preceded that of the erythrocytes. Also the presence of iron deficiency may be another contributory factor in the production of a low serum folate level. Chanarin (1969) believes that it may be wiser to disregard serum folate deficiency when not accompanied by a similar diminution in red cell folates, as the latter are not affected by minor fluctuations which may change serum folate levels. Herbert (1962) showed that a low folate intake may in a few days be followed

by a fall in serum folates to low levels because of the cessation of the exchange mechanism between absorbed and tissue folates.

The incidence of megaloblastic anaemia in kwashiorkor varies in different countries. In Durban, Walt, Holman, and Hendrickse (1956) reported megaloblastosis in 100% of their kwashiorkor cases, while Adams *et al.* (1967) in the same town found megaloblastic erythropoiesis in only 28% of cases. Sandstead *et al.* (1965) in Cairo reported an incidence of 35%. In Alexandria, Khalil *et al.* (1970) reported a megaloblastic bone marrow reaction in 52% of kwashiorkor cases and 43% of marasmics. In the present study, marrow megaloblastosis was shown in 45 and 37% of cases of kwashiorkor and marasmus, respectively. This high variability may be partially attributed to different interpretation of marrow smears showing early megaloblastic reactions by different haematologists. This personal factor may add to the validity of erythrocyte folate estimation as an accurate criterion for the diagnosis of folate deficiency (Kitay, 1969).

Table II shows the very close correlation between the bone marrow reaction and the erythrocyte folate level, as well as the much less close, but still highly significant, correlation between the marrow reaction and the serum folates. Only 5 of the malnourished infants had a marrow reaction different from that predicted on the basis of erythrocyte folates, while 12 had a reaction different from that predicted from the serum folates. On the other hand, there was no significant difference in the degree of nuclear segmentation of neutrophils between cases of malnutrition and their matching controls, possibly due to concomitant infection which was common among the former group of cases.

Megaloblastosis in protein calorie malnutrition may be largely attributed to deficiency of folate intake. The usual diet of the studied cases was mainly composed of small quantities of boiled cow's or powdered milk together with rice, which are poor in their folate content. They also used to have some potatoes and vegetable soup, which after prolonged cooking are usually deprived of a good percentage of their folate content. Layrisse *et al.* (1964) reported that in addition to the probability of dietary lack of folic acid in these patients, malabsorption, which they showed in a good percentage of their cases, may be a contributory factor. Diarrhoea, or a recent history of gastrointestinal disturbances, was common, among the cases of protein calorie malnutrition in this study.

Serum proteins were significantly low in kwashiorkor but not in marasmus. However, both groups

manifested a significant diminution of the mean corpuscular Hb concentration and a significant rise of the mean corpuscular volume presenting with a hypochromic macrocytic anaemia, due to a combined deficiency of iron and folic acid. Larsen (1948) stated that besides folate deficiency, macrocytosis may be due to fatty infiltration of the liver, which is a common finding in kwashiorkor. Macrocytosis was present in 54% of the studied cases of kwashiorkor and the rest were normocytic. On the other hand, 42% of the marasmus cases were macrocytic, 5% were microcytic, and the rest were normocytic.

Vitamin B₁₂ deficiency was reported to be infrequent in protein calorie malnutrition. However, those cases presenting with a megaloblastic anaemia responded to very small doses of the vitamin (Woodruff, 1969). Macdougall and Ross (1960), Adams and Scragg (1962), and Sandozai *et al.* (1963) found that vitamin B₁₂ deficiency rarely occurs in kwashiorkor. Even serum vitamin B₁₂ may be abnormally high in some cases and may drop to normal after treatment. The range of serum vitamin B₁₂ in the present group of normal controls varied between 194 and 768 pg/ml, with a mean of 393.4 pg/ml, while the mean values in cases of kwashiorkor and marasmus were 494 and 411.7 pg/ml, respectively, denoting no significant difference.

It can accordingly be stated that megaloblastic anaemia in protein calorie malnutrition was associated with folate and not with vitamin B₁₂ deficiency, and this was borne out by the more valid red cell folate assay as well as by the less conclusive serum folate assay. The normal serum vitamin B₁₂ levels found excludes the possibility that the low red cell folate was secondary to vitamin B₁₂ deficiency.

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