Hematologic Studies in Kwashiorkor

S. M. Perreira, M.R.C.P.(B)† and S. J. Baker, M.D.†

Children with kwashiorkor frequently have a moderate to severe degree of anemia, which has often been described as orthochromic and normocytic.1-4 Walt et al.5 and Scragg and Rubidge6 found in Durban that a significant number of patients (20 per cent) with kwashiorkor had megaloblastic changes in the bone marrow. Mehta and Gopalan7 reported that eleven of eighteen children with kwashiorkor had megaloblastic changes in their study at Coonoor, South India. Velez et al.8 in Columbia, recorded megaloblastic anemia in kwashiorkor, responsive to folie acid therapy. Of fifty children with kwashiorkor, Kho et al.9 in Djakarta found megaloblasts in the bone marrow in five and giant stab cells and hypersegmented polymorphonuclear leukocytes in several others.

It is apparent that the incidence of megaloblastic anemia in children with kwashiorkor varies from country to country. The present study was undertaken to establish the hematologic findings of children with kwashiorkor in Vellore, South India, and to compare the results with those of normal children of the same age and socioeconomic status.

MATERIAL AND METHODS

From September 1963 to June 1965 a hundred children with kwashiorkor, aged one to four years, were admitted to the Nutrition Research Unit. Two children were excluded from the study because of severe cachexia of the skin rendered bone marrow aspiration inadvisable, and three others died.

From the Nutrition Research Unit and Wellcome Research Unit, Christian Medical College Hospital, Vellore, South India.

* Reader in Pediatrics, Nutrition Research Unit; † Professor of Medicine, Wellcome Research Unit.

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within a few hours of admission. The remaining ninety-five children were included in the study.

All the patients exhibited the apathy and edema of the syndrome, and 80 per cent of them had the associated skin and hair changes.8 Many had overt manifestations of multiple vitamin deficiencies. Over half of them had the ocular signs of vitamin A deficiency, and one-third angular stomatitis.

On admission to the hospital and before treatment was initiated, venous blood was drawn, after a 5 hour fast, for hematologic investigations and serum protein estimations. Bone marrow was also obtained on the day of admission by iliac crest aspiration. All investigations, including those of bone marrow studies, were repeated at weekly intervals throughout the child's stay in the hospital.

Twenty-seven children in apparent good health and of comparable age and socioeconomic status served as controls; venous blood was drawn, after an overnight fast, for the same estimations.

The hematologic technics employed were those described by Dacie.10 Hemoglobin was estimated by the cyanmethemoglobin method using a photo-electric colorimeter calibrated and checked at regular intervals with a hemoglobin standard.11 Bone marrow smears were stained with May-Grunnwald-Giemsa stain and interpreted without any knowledge of the patient or the serum levels of iron, vitamin B12 or folic acid. Megaloblastic bone marrows were graded 1 to 4, depending on the severity of the morphologic change, the mildest degree being designated grade 1.12 Shrinkage smears were stained for iron with potassium ferrocyanide and known positive controls were included in each batch.

Serum vitamin B12 levels were estimated by the method of Ross et al.13 using Euglena gracilis, Z strain, and serum folate levels were measured using Lactobacillus casei as the test organism.13,14 Serum total proteins were determined by the microciniunet method and the protein fractions by paper electrophoresis at pH 8.6, staining with bromphenol blue and eluting the dye with 0.01 N sodium hydroxide.14

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from 6.4 to 7.5 gm. per 100 ml. (mean 7.04 gm.) and the serum albumin fractions from 3.0 to 4.4 gm. per 100 ml. (mean 3.61 gm.).

In the children with kwashiorkor, the serum total proteins ranged from 2.3 to 3.4 gm. per 100 ml., with a mean of 3.3 gm. All these children had low serum albumin levels with values ranging from 0.6 to 2.6 gm. per 100 ml. (mean 1.5 gm.).

**Hemoglobin**

In the control group, hemoglobin levels ranged from 0.9 to 14.8 gm. per cent with a mean of 11.5 gm. per cent. In three children (11 per cent) the hemoglobin level was below 10 gm. per cent.

In the children with kwashiorkor, a moderate degree of anemia was usual. The values ranged from 3.5 to 13.6 gm. per cent with a mean of 8.5 gm. Of the ninety-five children hemoglobin levels were below 10 gm. per cent, in sixty-nine (73 per cent) and below 7 gm. per cent in twenty-three (24 per cent) (Fig. 1). There was no correlation between the severity

**RESULTS AT THE TIME OF ADMISSION**

**Serum Proteins**

Serum proteins and fractions were estimated in eighteen of the twenty-seven children who served as controls. Serum total proteins ranged

![Diagram of Hemoglobin levels](image)

**Fig. 1.** Hemoglobin values in grams per cent of twenty-seven normal children and ninety-five children with kwashiorkor. The mean values and 1 standard deviation are indicated on the vertical lines.

After admission and throughout their stay in hospital, the children with kwashiorkor also participated in a feeding trial. They were given 50 to 100 gm. of skim milk or 75 to 170 gm. of fishflour, according to their body weight, as the main sources of protein, and rice and coconut oil to supply the necessary calories.

The following vitamin supplement was given every day: Vitamin A 10,000 I.U., ascorbic acid 100 mg., thiamine 0.5 mg., riboflavin 0.9 mg., niacin 5.0 mg., pyridoxine 0.3 mg. and calcium pantothenate 2.5 mg. No supplements of iron, vitamin B12 or folic acid were given until after at least one week's observation.

![Diagram of Serum Vitamin B12 levels](image)

**Fig. 2.** Serum vitamin B12 levels in control subjects and in the children with kwashiorkor. The solid circles denote the values of patients with megaloblastic bone marrow. Mean values and 1 standard deviation are indicated on the vertical lines.
of kwashiorkor, as judged by the serum albumin levels, and the degree of anemia as judged by the packed cell volume (r = 0.47).

**Serum Vitamin B₁₂**

The levels of serum vitamin B₁₂ in the control subjects ranged from 102 to 414 µg per ml, with a mean of 206 µg per ml. In the children with kwashiorkor the levels were in general higher, with a mean of 423 µg per ml and a range of 76 to 2000 µg per ml (Fig. 2). The difference between the groups was statistically significant (p < 0.001).

Eleven patients had serum vitamin B₁₂ levels of 1,000 µg per ml or more. Two had serum vitamin B₁₂ levels below 100 µg per ml. One of these had the hyperpigmentation of the skin over the knuckles and at the flexures that has been described in vitamin B₁₂ deficiency. ¹³

**Folic Acid**

In the control group serum folic levels ranged from 3 to 21 µg per ml with a mean of 11.5 µg per ml. Only one child had a folic level below 6 µg per ml.

In the children with kwashiorkor the range was from 1.4 to 25 µg per ml with a mean of 6.7 µg per ml; more than half had levels below 6 µg per ml (Fig. 3). The difference between the patients and the control subjects was statistically significant (p < 0.001).

**Serum Iron**

Serum iron was estimated in nineteen of the children who served as controls and in seventy-eight of the children with kwashiorkor (Fig. 4). The value in the control subjects ranged from 30 to 120 µg per 100 ml, with a mean of 72 µg per 100 ml; whereas in the children with kwashiorkor the values ranged from 12 to 110 µg per 100 ml, with a mean of 42 µg per 100 ml. There was some overlap of values in the two groups, but the children with kwashiorkor tended to have lower levels (p < 0.001).

**Bone Marrow**

On admission, fifty-eight of the ninety-five patients with kwashiorkor had a normoblastic bone marrow, and thirty-seven had a megaloblastic marrow.

Two-thirds of the patients with kwashiorkor
had cytologic evidence of iron deficiency in the developing red cells such as irregular or rugged cell margins and nonhemoglobinization of the cytoplasm in the late normoblasts. In thirty-nine patients, the bone marrow smears were stained for iron. In three iron was stainable but in the remaining thirty-six no stainable iron could be demonstrated.

In twenty-four of the thirty-seven children with megaloblastic bone marrows serum folic acid levels were below 6 µg. per ml.; one child had a serum vitamin B₉ level below 100 µg. per ml. and one had low levels of both vitamin B₉ and folic acid (Fig. 5). The remaining eleven had serum levels of vitamin B₉ and folic acid not usually associated with megaloblastosis (Table I).

**Seasonal Variation**

From September to February, children with normoblastic marrows at the time of admission were in predominance, whereas from March to August, a higher proportion of the children admitted had megaloblastic marrows (Table II).

**Course in Hospital**

**Anemia**

In thirty patients there was a significant increase in the degree of anemia with a fall of hemoglobin of 2 to 4 gm. per 100 ml. by the end of the first or second week of protein supplementation.

**Serum Vitamin B₉ Levels**

In the eleven children with vitamin B₉ levels of 1,000 to 2,000 µg. per ml., the levels fell steadily during hospitalization. By the end

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**Table I**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bone Marrow (grade)*</th>
<th>Vitamin B₉</th>
<th>Folic Acid</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. N.</td>
<td>1-2</td>
<td>200</td>
<td>10.0</td>
<td>Died</td>
</tr>
<tr>
<td>M. R.</td>
<td>3</td>
<td>288</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>K. I.</td>
<td>2</td>
<td>1,100</td>
<td>9.75</td>
<td></td>
</tr>
<tr>
<td>C. Y.</td>
<td>2</td>
<td>184</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>J. S.</td>
<td>3</td>
<td>800</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>S. A.</td>
<td>1-2</td>
<td>400</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>D. U.</td>
<td>2</td>
<td>300</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>S. R.</td>
<td>1-2</td>
<td>230</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>V. U.</td>
<td>1</td>
<td>328</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>K. R.</td>
<td>1-2</td>
<td>223</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>S. M.</td>
<td>1</td>
<td>192</td>
<td>14.0</td>
<td></td>
</tr>
</tbody>
</table>

* Megaloblastic bone marrows were graded as follows: 4 = severe; 3 = moderate to severe; 2 = moderate; 1 = mild.
of the third week, the values ranged from 180 to 750 μg. per ml. (Fig. 6).

**Serum Folate Levels**

On admission the serum folate levels were above 6 mg. per ml. in forty-one children. In fourteen of these the folate levels did not fall appreciably during hospitalization. In the other nineteen folate levels fell progressively to below 6 mg. per ml. In eighteen of these nineteen children, the bone marrow turned megaloblastic or became more severely megaloblastic as the serum folate levels decreased.

**Fig. 5.** Serum vitamin B<sub>12</sub> and folate acid levels in children with kwashiorkor and megaloblastic bone marrows. Eleven children had megaloblastic marrows with serum vitamin B<sub>12</sub> levels above 100 μg. per ml. and serum folate levels above 6 mg. per ml.

**Bone Marrow Morphology**

In twenty-one of the children with normoblastic marrows on admission megaloblastic changes developed during hospitalization. In twelve of these the change followed protein repletion and iron therapy given to correct an iron deficiency. In the remaining nine the megaloblastic change occurred during protein repletion prior to the administration of iron, but in three of these the megaloblastosis subsequently became more pronounced when iron was administered.

**Fig. 6.** The change in serum vitamin B<sub>12</sub> levels in eleven children with kwashiorkor on admission and during their stay in hospital.

**Children with "Unexplained" Megaloblastosis**

Of the eleven children with megaloblastic bone marrows and apparently normal serum vitamin B<sub>12</sub> and folate levels, one died a few days after admission. In seven the serum folate level fell to below 6 mg. per ml. at the end of the first or second week (Fig. 7). In the remaining three the serum folate level did not
fall significantly. However, these three children responded to folate acid therapy, either oral or parenteral.

Response to Treatment

Patients in whom a significant degree of anemia developed were treated with appropriate hematines. Treatment with ferrous sulfate (50 mg. per day), folic acid (25 mg. per day) and vitamin B12 (0.1 mg. per day) resulted in satisfactory hematologic responses in all cases. Usually the small doses of vitamin B12 and folic acid were given orally and in every case produced satisfactory responses indicating normal intestinal absorption of these substances.

COMMENTS

The incidence of megaloblastic change in the bone marrow at the time of admission in this series of ninety-five children with kwashiorkor was 39 per cent. In an additional 22 per cent megaloblastosis developed during their stay in the hospital making the over-all incidence of megaloblastosis 61 per cent of the cases. The majority appear to be associated with folate deficiency.

A greater proportion of patients had megaloblastic bone marrows on admission during March to August than during the other months of the study. In the rural community from which the patients came, negligible quantities of animal foods are eaten for economic reasons, and folate requirements are almost entirely supplied by green leafy vegetables. During the dry hot months of March to June and the four to six weeks immediately following the onset of the rains, green leafy vegetables are in short supply. The varying incidence of megaloblastosis, therefore, probably reflects the seasonal dietary folate deficiency. Walt et al. have described a similar variation in the incidence of megaloblastic anemia in malnourished children in South Africa.

The eleven children with megaloblastic bone marrows and apparently normal serum vitamin B12 and folate levels are of interest. One obvious explanation for this phenomenon is that the folate levels were due to faulty assay. However, from the study of other patients by the same assay, at the same time, this does not seem likely. The levels of serum vitamin B12 were not estimated nor was the therapeutic effect of vitamin B12 tried. We do not know, therefore, whether these eleven children were comparable to the patients described by Majaj et al., who apparently responded to vitamin B12 therapy. In three children of this group, the bone marrow became normoblastic when given folic acid orally although serum folate levels were in the normal range. In seven others not given folic acid as part of their therapy serum folate levels subsequently dropped to levels below 6 mg. per ml. on protein feeding. The results suggest that protein depletion may in some way interfere with folate metabolism, causing an accumulation of L. casei-active material in the serum, in a form not available to the developing red cell. This subject is at present under further study.

We are aware of the reports of hypoplasia and aplasia of the bone marrow in children with malnutrition. In our series of ninety-five patients, only one child exhibited hypoplasia of the bone marrow. This child was seriously ill with a staphylococcal pneumonia at the time of admission and had severe hypoplasia of the marrow which became normoblastic as the infection responded to tetracycline. In none of the patients was an aplasia of the bone marrow found during the period of recovery from kwashiorkor as described by Foy and Kondi.

One-third of the children showed a decrease in hemoglobin of 2 to 4 gm. per cent during hospitalization. In some, the fall occurred when the child was still frankly edematous; in others, the fall occurred when the child was free of edema and gaining weight. It was difficult, therefore, to ascribe the decrease in hemoglobin to changes in blood volume occurring as edema cleared. The reduction in hemoglobin did not appear to be due to a fall in folate levels, for thirteen of the thirty showed an associated decrease in folate whereas in seventeen others the serum folate level remained high. Trowell and Simpkins noted that the anemia, which was ascribed to protein deficiency, did not diminish when the children were given a high
protein diet. They recorded, as did others, a fall in hemoglobin levels during the first week of hospitalization and noted that the decrease in hemoglobin could be prevented by the intramuscular administration of iron dextran. No attempt was made in this study to confirm their findings.

Extremely high levels of serum vitamin B12 have been reported by Sato et al.23 in their cases of protein malnutrition. They correlated the high levels with the degree of fatty change in the liver, and noted lowering of the serum levels of vitamin B12 and disappearance of fat from the liver during recovery.

Serial liver biopsies were not undertaken in the present study, but from previous experience, we know that even though there is marked improvement in the degree of fatty infiltration, not all the fat has cleared from the liver by the time the serum vitamin B12 levels approach normal values, i.e., in two to four weeks time. The fall from excessively high levels to normal levels is probably a reflection of improving liver function with consequent improvement in the liver "storage" of the vitamin.

SUMMARY

The hematological findings in ninety-five children with kwashiorkor are described and compared with those in twenty-seven apparently normal control subjects of the same age and socioeconomic status.

The children with kwashiorkor as a group had a moderate degree of anemia, low serum iron, low serum folate and high serum vitamin B12 levels.

Megablasticosis of the bone marrow was found in a total of thirty-seven (39 per cent) children with kwashiorkor at the time of admission and developed in an additional twenty-one (22 per cent) during their stay in hospital. There was a seasonal variation in the incidence of megablasticosis:

Twenty-four of the thirty-seven children with megablastic bone marrow at the time of admission had low levels of serum folate, and two a low level of serum vitamin B12. The remaining eleven had apparently normal levels of serum B12 and folate.

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