Evaluation of erythropoiesis in protein energy malnutrition

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تقييم تكوُّن الكريات الحمر في سوء التغذية بالبروتين والطاقة

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الخلاصة: قُيِّمت الدراسة تكوُّن الكريات الحمر لدى 50 رضيعاً أُدخلوا المستشفيات لإصابتهم بسوء التغذية بالبروتين والطاقة، ولدى 50 رضيعاً شاهداً. وقد وجد لدى الرضع المصابين بسوء التغذية بالبروتين والطاقة أن تعداد الكريات الحمر وهيموغلوبين الكريات الوسطي ومُتَوَسِّط الكريات الحمر بكرية أقل بدرجة يُعتدُّ بها إحصائياً مما لدى الرضع الشواهد، في حين كان تعداد الكريات البيض ومتوسط هشاشة الكريات وعرض توزع الكريات الحمر أكبر بكثير لدى الرضع المصابين بسوء التغذية بالبروتين والطاقة لدى إدخالهم المستشفيات مما لدى الرضع الشواهد؛ فيما كانت بروتينات المصل الكلية والألبومين وسكر الدم على الريق وفولات المصل أقل بكثير عند الرضع المصابين بسوء التغذية بالبروتين والطاقة لدى إدخالهم المستشفيات مما لدى الرضع الشواهد؛ أما عند التخرج من المستشفى فقد كان فيريتين المصل أعلى بكثير والسعة الكلية لربط الحديد أقل بكثير لدى الرضع المصابين بسوء التغذية بالبروتين والطاقة مما لدى الرضع الشواهد. وقد كان إريثروبيوتين المصل لدى الرضع المصابين بسوء التغذية بالبروتين والطاقة أعلى بكثير مما لدى الرضع الشواهد وقت إدخالهم ووقت تخريجهم من المستشفيات. إن فقر الدم الناجم عن عوز البروتين والطاقة ينتج عن عوز مركب يؤدي إلى تعطيل تكوين الكريات الحمر بسبب عدم استجابة نقي العظم لزيادة مستوى الإريثروبيوتين.

ABSTRACT This study evaluated erythropoiesis in 50 infants hospitalized with protein energy malnutrition and in 50 control infants. The red cell count, mean corpuscular haemoglobin and reticulocyte index were significantly lower, while the white blood cell count, median corpuscular fragility and red cell distribution width were significantly higher on admission than in controls. Total serum protein, albumin, fasting blood glucose, and serum folate were significantly lower on admission than in controls. Serum ferritin was significantly higher and total iron-binding capacity was significantly lower on discharge compared to controls. The serum erythropoietin was significantly higher on admission and discharge than in controls. The anaemia of protein energy malnutrition is due to mixed deficiencies resulting in ineffective erythropoiesis despite an increased level of erythropoietin.

Evaluation de l'érythropoïèse dans la malnutrition protéino-énergétique

RESUME La présente étude a évalué l'érythropoïèse chez 50 nourrissons hospitalisés pour malnutrition protéino-énergétique et chez 50 nourrissons témoins. La numération érythrocytaire, l'hémoglobine globulaire moyenne et l'index réticulocytaire étaient significativement moins élevés, tandis que le chiffre des leucocytes, la fragilité corpusculaire médiane et l'indice de distribution érythrocytaire étaient significativement plus élevés lors de l'admission à l'hôpital que chez les témoins. Les protéines sériques totales, l'albumine, la glycémie à jeun et les folates sériques étaient significativement moins élevés lors de l'admission que chez les témoins. La ferritine sérique était significativement plus élevée et la capacité de fixation du fer était significativement moins élevée à la sortie d'hôpital que chez les témoins. L'érythropoïétine sérique était significativement plus élevée lors de l'admission et à la sortie d'hôpital que chez les témoins. Dans la malnutrition protéino-énergétique, l'anémie est causée par diverses carences qui entraînent une érythropoïèse inefficace malgré une élévation du niveau d'érythropoïétine.

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Introduction

According to the World Health Organization (WHO), 149.6 million children under 5 years of age, i.e. 26.7% of the world's children in this age group, are malnourished in terms of weight for age. Geographically, over two-thirds (72%) of the world's malnourished children live in Asia, while 25% are found in Africa and 2.3% in Latin America [*1*]. Furthermore, malnutrition is an accomplice in at least half of the 10.9 million child deaths each year [*1*]. Protein energy malnutrition (PEM) is by far the most lethal form of malnutrition and is one of the leading causes of morbidity and mortality in children [*2*]. PEM probably contributes to the deaths of 5 million children each year [*3*]. Many classifications of malnutrition have been used over the past 30 years. The WHO has published a new classification for PEM called the Z-score or standard deviation classification of malnutrition [*4*].

PEM is a generalized disorder affecting the structure and function of the entire body. Changes in the haematological system, including anaemia, are an invariable feature of the syndrome. Anaemia may be normochromic or hypochromic, normocytic, macrocytic or microcytic. Many important questions about this problem remain unanswered and new ones continue to emerge [*5*].

The production of red blood cells (RBCs) is governed by various growth factors. Of all the factors stimulating erythropoiesis none has a more important regulatory role than erythropoietin. Erythropoietin binds to specific receptors on the erythroid bone marrow precursors [colony forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E)], thus stimulating their differentiation and clonal maturation into mature RBCs [*6*]. Other requirements for normal erythropoiesis are protein, iron for haemoglobin synthesis, vitamin B_{12} and folate for DNA synthesis, as well as other vitamins such as pyridoxine, riboflavin, thiamine, vitamins C and E, and trace elements such as cobalt [*7*].

The aim of the present work was to evaluate some important erythropoietic factors in hospitalized infants diagnosed with PEM, on admission to hospital and on their discharge following nutritional recovery. These factors might be of value in identifying the type of anaemia associated with PEM and in planning a therapeutic regimen.

Methods

Fifty (50) infants diagnosed with PEM and admitted to the University of Alexandria Children's Hospital (El-Chatby) for a mean duration of 12 days, and fifty age- and sex-matched healthy infants as controls were included in this study. Informed consent was obtained from all parents and the University of Alexandria Ethical Committee approved the protocol of the study.

On admission, all cases were diagnosed and classified according to the WHO new classification of PEM by Z-score or standard deviation classification of malnutrition [*4*]. None of the children enrolled in the study had any apparent clinical infection. A thorough history was taken and detailed physical examination, emphasizing any signs of malnutrition or vitamin deficiencies, performed. Patients were stabilized on admission, complications were treated, and fluid and electrolyte imbalances corrected. Thereafter, nutritional rehabilitation was started. The dietetic therapy was administered orally or by nasogastric tube as needed. The initial diet provided 75 kcal/100 mL and < 1 g protein/100 mL given as 100 mL/kg body weight per day in 10 small feeds.

On return of appetite the infant was provided with 100 kcal/100 mL and 2.5–3 g protein/100 mL, given as 120–130 mL/kg body weight per day in 8 feeds. Multivitamin preparations were given to provide double the recommended daily allowance. No iron preparations were given during hospitalization.

The criteria for discharge from the hospital were changed mood, increasing appetite, cessation of diarrhoea, disappearance of oedema (if present), initiation of weight gain, and a serum albumin level > 3 g/dL. After discharge patients were followed up at the nutrition clinic on a weekly basis.

The following laboratory tests were performed on a morning blood sample taken from both patients and controls: complete blood picture with differential count and indices, total serum protein and albumin, reticulocyte index, serum ferritin by enzyme-linked immunoassay using Eurogenetics kits, serum iron and total iron binding capacity (TIBC) by quantitative determination using Teco Diagnostics kits, serum folate by radioimmunoassay using Dia Sorin Kits, serum erythropoietin by radioimmunoassay using Diagnostic Products Corporation kits, blood urea and serum creatinine (to evaluate renal function), and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (to evaluate liver function). Serum was separated and kept refrigerated at –70 °C until all tests were performed. Tests were repeated for patients on discharge. A plain chest X-ray, and complete urine and stool analyses were also performed for all children.

Epi-Info was used for data presentation and statistical analysis of the results. The following statistical measures were used:

- Descriptive measures included count, percentage, arithmetic mean, standard deviation, and range.
- Statistical tests included the chi-squared test for analysis of qualitative variables, Student *t*-test and one-way analysis of variance (*F*), and Scheffe test (for pairwise comparison) were used for quantitative variables.

The level of significance selected for this study was $P \leq 0.05$.

Results

The main presenting symptoms of the study population were apathy and restlessness in 84%, anorexia in 72% and oedema in 36%; the main signs were muscle wasting in 100%, loss of subcutaneous fat in 56%, pallor in 68%, dermatitis in 32%, hypothermia in 20%, hair changes in 16%, keratomalacia in 4%, angular stomatitis in 40% and glossitis in 8%.

Table 1 shows the characteristics and anthropometric measurements of infants with PEM on admission to hospital, compared to those of the controls. There were no significant differences in the age or sex distribution of cases and controls (7.4 ± 4.0 versus 7.45 ± 3.4 months, 21 males and 29 females versus 20 males and 30 females respectively). There were statistically significant differences between children with PEM on admission and controls in weight, length, head circumference and mid-arm circumference. The percentage weight change in children with PEM from admission until hospital discharge varied from –2.5% to 25.6%.

Table 2 shows the blood picture of infants with PEM on admission compared to controls. It shows that the RBC counts and mean corpuscular haemoglobin (MCH) of children with PEM were significantly lower than controls. The white blood cell (WBC) count, platelet count, median corpuscular fragility (MCF) and red cell distribution

Table 1 **Characteristics and anthropometric measurements of infants with PEM on admission and controls**

Variable	Infants with PEM ($n = 50$)	Controls ($n = 50$)	Statistical data
Age (months)			
Range	2.0–18.0	2.0–17.0	$t = 0.0674$
Mean ± *s*	7.4 ± 4.0	7.5 ± 3.4	$P = 0.9464$
Sex			
Male	21 (42%)	20 (40%)	$\chi^2 = 0.0832$
Female	29 (58%)	30 (60%)	$P = 0.7729$
Weight on admission (kg)			
Range	2.2–7.5	4.0–12.5	$t = 9.653$*
Mean ± *s*	4.6 ± 1.4	7.9 ± 1.9	$P < 0.001$
Weight on discharge (kg)			
Range	2.7–8.0		
Mean ± *s*	5.0 ± 1.4		
% weight change[a]			
Range	–2.5 to 25.6		
Mean ± *s*	6.8 ± 6.7		
Length (cm)			
Range	49.0–77.0	59.0–86.0	$t = 6.267$*
Mean ± *s*	62.0 ± 7.2	71.9 ± 8.5	$P < 0.001$
Head circumference (cm)			
Range	33.0–47.00	37.0–47.0	$t = 3.98$*
Mean ± *s*	40.3 ± 3.6	42.8 ± 2.7	$P < 0.001$
Mid-arm circumference (cm)			
Range	6.0–14.0	9.00–17.00	$t = 8.40$*
Mean ± *s*	9.0 ± 2.6	13.2 ± 2.4	$P < 0.001$

[a] *% weight change from admission until hospital discharge.*
**Significant at P < 0.05.*
PEM = protein energy malnutrition.
s = *standard deviation.*

width (RDW) values were significantly higher on admission in children with PEM than in controls. There were no significant differences between the groups in mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

Table 3 shows the nutritional and laboratory data of infants with PEM on admission and following recovery compared to controls. The mean duration of admission to the hospital was 12.2 ± 4.8 days. The nutritional data revealed that total serum protein and serum albumin were significantly lower on admission compared to controls and that serum protein level on discharge was still significantly lower than in controls. Blood urea and serum creatinine were significantly higher on admission and on discharge compared to controls, with the admission levels significantly higher than those found on discharge. ALT val-

Table 2 **Blood picture of infants with PEM on admission and controls**

Variable	Infants with PEM on admission ($n = 50$)	Controls ($n = 50$)	Statistical data
Red blood cell count ($\times 10^6$ cells/mm^3)			
Range	1.9–4.6	3.2–5.1	$t = 6.82$*
Mean ± s	3.2 ± 0.7	4.1 ± 0.5	$P < 0.001$
White blood cell count ($\times 10^3$ cells/mm^3)			
Range	5.1–19.5	4.0–10.4	$t = 8.74$*
Mean ± s	12.6 ± 3.8	7.5 ± 1.7	$P < 0.001$
Platelet count ($\times 10^3$cells/mm^3)			
Range	135.0–600.0	120.0–628.0	$t = 3.11$*
Mean ± s	350.0 ± 118.0	277.4 ± 115.9	$P < 0.001$
MCV (fl)			
Range	70.3–93.0	74.0–88.0	$t = 1.486$
Mean ± s	80.3 ± 5.9	81.8 ± 4.0	$P = 0.1404$
MCH (pg/cell)			
Range	17.0–33.0	24.0–33.1	$t = 3.11$*
Mean ± s	27.1 ± 3.9	29.1 ± 2.5	$P < 0.001$
MCHC (g Hb/dL RBC)			
Range	27.5–38.3	30.0–37.7	$t = 1.12$
Mean ± s	33.7 ± 2.8	34.3 ± 2.1	$P = 0.264$
MCF (% saline concentration)			
Range	0.45–0.6	0.45–0.5	$t = 5.000$*
Mean ± s	0.5 ± 0.5	0.40 ± 0.1	$P < 0.001$
RDW			
Range	13.6–30.0	11.8–14.2	$t = 11.284$*
Mean ± s	18.9 ± 3.8	12.2 ± 1.8	$P < 0.001$

**Statistically significant at P < 0.05.*
PEM = protein energy malnutrition.
MCV = mean corpuscular volume.
MCH = mean corpuscular haemoglobin.
MCHC = mean corpuscular haemoglobin concentration.
MCF = median corpuscular fragility (50% lysis osmotic fragility).
RDW = red cell distribution width.
s = *standard deviation.*

ues were comparable in patients (both on admission and on discharge) and in controls. Fasting blood glucose levels were significantly lower on admission than on discharge and in controls. The haematological parameters revealed that there was no significant difference in serum iron between the three groups, while serum ferritin was significantly higher and TIBC significantly lower on discharge compared to controls. Serum folate was significantly lower on admission compared to the con-

Table 3 **Nutritional and laboratory data of infants with protein energy malnutrition (PEM) on admission and on discharge, and controls**

Variable	Infants with PEM on admission	Infants with PEM on discharge	Controls	Statistical data
Total protein (g/dL)				
Range	3.3–7.0	4.3–7.5	6.8–8.1	$F = 93.9236^*$
Mean ± *s*	5.3 ± 0.9[a]	6.1 ± 0. 9[a,b]	7.4 ± 0.4	$P < 0.001$
Albumin (g/dL)				
Range.	1.0–4.5	3.0–5.2	3.1–4.5	$F = 83.349^*$
Mean ± *s*	2.0 ± 1.0[a]	3.7 ± 0.8[b]	3.8 ± 0.4	$P < 0.001$
Blood urea (mg/dL)				
Range	12.0–40.0	9.0–32.0	5.0–18.0	$F = 22.4699^*$
Mean ± *s*	20.2 ± 7.3[a]	17.1 ± 5.0[a,b]	12.7 ± 4.0	$P < 0.001$
Serum creatinine (mg/dL)				
Range	0.4–1.0	0.3–0.8	0.1–0.8	$F = 14.2272^*$
Mean ± *s*	0.6 ± 0.2[a]	0.5 ± 0.1[a,b]	0.4 ± 0.2	$P < 0.001$
ALT (U/L)				
Range	19.0–40.0	18.0–34.0	20.0–35.0	$F = 1.1428$
Mean ± *s*	28.1 ± 9.4	26.5 ± 8.8	25.7 ± 5.6	$P < 0.3217$
ALP (U/L)				
Range	8.0–34.0	7.0–20.0	7.0–15.0	$F = 22.0634$
Mean ± *s*	15.8 ± 6.0[a]	11.1 ± 4.1[b]	10.3 ± 2.3	$P < 0.001$
Serum iron (mg/dL)				
Range	30.0–140.0	52.0–156.0	69.0–120.0	$F = 0.87419$
Mean ± *s*	88.8 ± 29.8	95.0 ± 26.0	89.9 ± 17.9	$P = 0.419356$
TIBC (mg/dL)				
Range	130.0–470.0	140.0–480.0	170.0–420.0	$F = 3.91322^*$
Mean ± *s*	256.3 ± 108.8	234.6 ± 90.8[a]	285.8 ± 72.4	$P = 0.0220$
Serum ferritin (ng/mL)				
Range	30.0–680.0	40.00–920.00	40.00–217.00	$F = 19.0720^*$
Mean ± *s*	263.2 ± 169.0	317.5 ± 251.9[a]	103.0 ± 76.5	$P < 0.001$
Folate (ng/mL)				
Range	0.4–14.2	0.5–15.0	2.1–11.0	$F = 3.90982^*$
Mean ± *s*	2.74 ± 3.1[a]	3.7 ± 4.1	4.58 ± 2.5	$P = 0.02216$
Erythropoietin (mU/mL)				
Range	7.5–200.0	13.0–240.0	10.4–101.0	$F = 6.244^*$
Mean ± *s*	81.2 ± 45.6[a]	89.7 ± 69.9[a]	55.1 ± 28.9	$P = 0.0025$
Fasting blood glucose (mg/dL)				
Range	10.0–40.0	50.0–75.0	60.0–80.0	$F = 450.711^*$
Mean ± *s*	26.4 ± 7.7[a]	60.3 ± 6.8[a,b]	65.8 ± 6.8	$P < 0.001$
Haemoglobin (g/dL)				
Range	3.0–12.6	7.5–11.4	9.5–14.0	$F = 40.449^*$
Mean ± *s*	8.6 ± 2.1[a]	9.1 ± 2.5 [a]	12.0 ± 1.3	$P < 0.001$

Table 3 **Nutritional and laboratory data of infants with PEM on admission and on discharge, and controls** *(concluded)*

Variable	Infants with PEM on admission	Infants with PEM on discharge	Controls	Statistical data
Reticulocyte index				
Range	0.6–0.8	0.7–1.0	0.9–1.6	$F = 54.1216$*
Mean ± *s*	0.7 ± 0.2[a]	0.8 ± 0.20[b]	1.1 ± 0.2	$P < 0.001$

[a] *Significantly different from the control group.*
[b] *Significantly different from the group on admission.*
* *Statistically significant at* $P < 0.05$.
ALT = alanine aminotransferase. TIBC = total iron-binding capacity. ALP = alkaline phosphatase.

trols. Serum erythropoietin was significantly higher and haemoglobin concentrations were significantly lower on admission and on discharge compared to controls.

The reticulocyte index was significantly lower on admission and on discharge compared to controls; and the level on admission was significantly lower than the level on discharge.

Discussion

The etiology of anaemia in PEM has been attributed to a number of factors including deficiencies, infections, blood loss, haemolysis, erythroid hypoplasia and adaptation to lower metabolic oxygen requirements [8]. In our study, the red cell count, haemoglobin and MCH in patients with PEM were all significantly lower than in the controls. Such findings indicate that the anaemia in PEM is normocytic and normochromic, a finding that has been recorded in the majority of complicated cases of PEM in other studies [5]. Moreover, the presence of a significantly higher RDW among PEM patients in our study would indicate that the anaemia in our patients was due to mixed deficiencies, and/or early folate or iron deficiency, and/or erythroid hypoplasia, and/or infection [9].

Serum proteins, albumin and folate values of patients with PEM before treatment were significantly lower compared to controls. Any folate deficiency would be in the early stages, since late-stage deficiency would lead to macrocytic cells with increased MCV [9], which was not observed in our study. Protein and albumin have been shown to be important factors in the pathophysiology of anaemia in PEM [5] They directly affect the marrow erythroid activity and decrease haemoglobin by about 20%, as well as affecting TIBC by reducing its protein components. This was observed in our study since TIBC was significantly higher after establishment of nutritional rehabilitation and before hospital discharge of patients with PEM, with concomitant significant increase in total proteins and serum albumin. PEM also leads to a decrease in body lean mass and reduction of metabolic demand, and hence an "adaptation" to decreased active tissue mass by reduction in haemoglobin and total red cell mass [5].

Although the absence of stainable iron by bone marrow examination is generally considered the definitive marker of iron deficiency, we did not perform bone marrow examination as it is an invasive technique and the tests we used for assessing total

body iron stores (serum iron, TIBC and serum ferritin) accurately reflect normal iron stores.

According to various reports the incidence of iron deficiency in PEM is extremely variable [5,8]. Supplementation with iron during the nutritional management of PEM is of real importance only in proven cases of iron deficiency [5], which was not the situation in our study.

The median corpuscular fragility (osmotic fragility) was significantly higher in the PEM patients than in the controls, indicating the presence of an increased red cell deformability making them more vulnerable to haemolysis. This finding might be explained by the possible presence of metabolic changes in the red cell membrane, such as shortage of glucose RBC metabolic activities, as well as alterations in membrane lipids [5]. We found there was a significant reduction in blood glucose level in the patients with PEM before the start of treatment.

The interplay between PEM and infection remains the most important element in the morbidity and mortality attributed to childhood anaemia in Africa [10]. In our study there was a significant rise in leukocyte count in the patients with PEM compared to the controls, despite the fact that infants enrolled in the study did not show apparent clinical infection. PEM has been reported to act synergystically with infection, either clinically or subclinically. Infection leads to anaemia by reducing red cell survival, impairing iron bioavailability and impairing the response to erythropoietin [5].

Erythropoietin has been used in treatment of infants with anaemia [11]. However, we did not need to do so as the serum erythropoietin was significantly higher in our PEM patients (before treatment) than in controls, and became higher after treatment. Although haemoglobin increased from 8.6 to 9.1 g/dL after treatment, erythropoietin remained significantly elevated. Normally there is an inverse relationship between serum erythropoietin and haemoglobin concentration [12]. However in PEM there was no such physiological relationship. Moreover, the peak reticulocytic response did not occur over the mean hospitalization period of 12 days in our study, where the reticulocyte index did not rise significantly. Other investigators have showed that in PEM patients, erythropoietin remains elevated for a period of 4 weeks after starting treatment and then returns to normal levels [5]. Explanations suggested for the prolonged elevation of serum erythropoietin and the slow recovery of the anaemia in PEM are protein deficiency combined with rapid growth making the response to erythropoietin unsatisfactory [13]. In addition, sufficient iron must be present for a proper response to erythropoietin. In PEM the need for nutritional protein to synthesize erythrocyte haemoglobin by erythropoietin may produce a more general protein deficiency at a time when supply may be marginal. The ultimate result is a state of erythroid hyporesponsiveness to the normal or increased erythropoietin levels detected in PEM patients [5].

Other investigators, who have also found normal erythropoietin production in PEM, have suggested that the impairment of erythropoiesis in PEM could be due to an abnormality in the erythroid progenitor cell pool [14]. It has been shown that in anaemia of some chronic diseases there could be a blunted serum erythropoietin response to anaemia [15], which was not the situation in the anaemia of PEM infants in our study, where the erythropoietin response to anaemia was preserved.

In summary, the anaemia of PEM in our study was normocytic normochromic with high RDW. It could be attributed to mixed deficiencies with a high serum erythropoietin level. The failure of raised serum erythropoietin to induce an adequate increase of erythropoiesis may be due either to a reduced responsiveness of marrow erythropoietin-sensitive cells or to ineffective erythropoiesis compounded by mixed deficiencies.

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