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HYPERSEGMENTED NEUTROPHILS AND GIANT MYELOCYTES IN PROTEIN CALORIE MALNUTRITION

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Abstract

The qualitative leukocyte changes suggestive of erythroid maturation factor (folates, vitamin B_{12}) deficiency as evidenced by the presence of giant neutrophilic bands and metamyelocytes, a high lobe average, higher percent of the hypersegmented neutrophils are frequently observed in PCM children. Their significance and relationship with the deficiency state of iron, follates, vitamins B_{12} and E were investigated. It is concluded that although those leukocyte changes are the well known signs of megaloblastic anemias their presence in children with PCM are among the most common findings and apparently have no relationship with the vitamin B_{12}, vitamin E or folate deficiency. The results suggested that it may related to the associated infections and iron deficiency.

INTRODUCTION

Protein calorie malnutrition (PCM) is the most prevalent disease of nutritional deficiency in the world. (1) Although it affects primarily the young children, older children and adults are not spared. (2,3) From the earlist clinical description of PCM, pallor and anemia have been among the chief signs described. (4,5,9) Since then many descriptions have appeared in the literature. (4,5,6,7) In the majority of uncomplicated PCM the anemia observed are of normochromic normocytic type. However, the peripheral smear very often shows anisocytosis, poikilocytosis, occasional target cells with normal white cells and platelet counts. (8-21) Many

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investigators also observed PCM children with hypochromic microcytic anemia. (14-16, 18, 19, 22, 23) Macrocytic anemia is a prominent part of PCM in certain parts of the world (3, 11,12,14,15,18,21,24-27) such as in India, (14,21) Egypt, (19) South America, (26) and Africa. (27)

PCM is still one of the major pediatric problem in Northern Thailand. (28) Our previous studies (23) indicated that the etiologies of anemia in these children are protein and iron deficiencies although the striking leukocytic changes known to be associated with the erythroid maturation factor (EMF) deficiencies (esp. folic acid, vitamin B_{12}) were noted, This included the presence of hypersegmented neutrophilic leukocytes in blood smear, the observation of large proerythroblasts and giant metamyelocytes in bone marrow smears.

This is the analysis of the investigation of the significance of the above-mentioned leukocytic changes in relation to the abnormalities in the folic acid, vitamin B_{12}, vitamin E and iron status observed in children with PCM.

MATERIALS AND METHODS

Twenty two children between 1 - 3 year of age with clinical evidence of severe PCM according to Gomez's criteria (29) and a hemoglobin of less than 10.2 gm/100 ml. were admitted to the Pediatric Ward of Chiang Mai St. Louis Research Center. They were divided into 4 study group. The twelve week protocol was consisting of 2 six-week periods as follow.

SCHEDULE FOR SUPPLEMENT HEMATINICS

	FIRST 6 WEEKS	SECOND 6 WEEKS
GROUP I	None	None
GROUP II	Iron + Vit. E	Iron + Vit. E
GROUP III	Vit. E	Iron + Vit. E
GROUP IV	Iron	Iron + Vit. E

Whole blood hemoglobin levels were determined by the method of crosby, (30) microhematocrit was done by the method of McGovern et al. (31) White cell count and platelet count were carried out by the standard technics. (32,33) Reticulocyte counts were done with the New methylene blue method. (34) Bone marrow aspiration from the spinous proceses were carried out at interval. Defferential cell counts were done on the bone marrow and peripheral blood smears stained with Wright's stain. The stained peripheral blood smears were examined carefully for the petcentage of hypersegmented neutrophils, lope counts and lope averages. (35) Bone marrow smears prepared by squash technic were stained for hemosiderin and stainable liron with the Prussian Blue stain (36) then scored according to the criteria of Rath and Finch (37) Agar gel electrophoresis of hemoglobins was done by the method of Yakulis et al. (38) Heinz body preparations were made utilizing methyl violet in saline. (39) Glucose-6-phosphate dehydrogenase levels were also estimated. (40) Blood volume, red cell mass and 51 Cr. red cell survival were measured at interval. (41) Serum folates, (42) vitamin B_{12}, (43) vitamin E, (44) serum iron and total iron binding capacity (45) were measured at intervals.

TABLE I: LABORATORY FINDINGS ON ADMISSION

No.	Diagnosis	Hb. gm/100 ml	Serum Fe	TIBC	Folate	B_{12}	Vit. E	Hyper seg. %	Lope average	B.M. giant myeloid	B.M. Stainable Iron
1	MK	9.9	40	208	–	720	0.40	8	3.3	+	+
2	K	7.4	28	70	3.8	1860	0.10	2	2.3	+	0
3	K	7.9	46	63	3.4	–	0.10	1	2.4	+	±
4	K	5.7	46	51	2.7	658	0.10	1	2.9	+	+
5	M	9.7	62	305	5.6	–	0.10	0	2.6	0	0
6	MK	5.9	30	165	8.1	–	0.24	2	2.9	+	+++
7	M	9.8	38	232	7.8	825	0.30	0	2.3	+	+
8	M	9.6	26	135	2.0	2620	0.35	0	2.1	++	+++
9	K	8.6	100	102	5.5	346	0.10	0	2.6	0	+
10	MK	8.0	52	268	12.5	3430	0.10	4	2.9	++	0
11	M	9.7	74	125	14.0	1030	0.10	6	2.8	+	++
12	M	10.1	46	380	16.7	–	0.43	0	2.8	0	±
13	MK	7.5	46	136	3.4	1290	0.22	2	2.9	++	+++
14	M	10.0	44	196	5.0	570	0.10	0	2.3	++	+
15	K	9.2	66	72	4.7	1121	0.10	3	3.2	+	++
16	K	9.8	112	186	5.5	1582	0.10	5	2.8	+	±
17	MK	8.9	95	213	1.8	238	0.10	5	2.6	++	±
18	MK	9.0	58	98	3.0	–	0.27	0	2.5	0	±
19	K	9.8	96	115	3.5	331	0.15	2	2.7	+	0
20	MK	9.6	34	187	2.0	2075	0.30	1	2.5	++	+
21	M	10.0	52	227	5.5	743	0.20	8	3.0	+	±
22	K	9.6	100	176	3.0	480	0.15	6	3.3	+	+

NOTE

– Serum Fe, TIBC and vitamin E levels are reported in mg/100 ml.

– Serum folate levels are reported in ng/ml.

– Serum vitamin B_{12} levels are reported in pg/ml.

TABLE II: LABORATORY FINDINGS AFTER RECOVERY FROM PCM

No:	Hb.	Serum Fe	TIBC	Folate	Vit. B_{12}	Vit. E	Hyperseg %	Lobe average	B.M. giant myeloid	B. M. Stainable iron
1	11.0	58	484	27.5	537	0.40	6	2.9	0	0
2	11.3	94	419	15.6	733	0.70	2	3.1	0	0
3	8.9	24	420	32.0	762	0.50	5	2.9	0	0
4	10.7	54	238	42.0	447	0.40	5	3.3	+	0
5	7.9	37	450	24.0	–	0.37	7	3.2	0	0
6	10.1	56	420	28.0	–	0.52	2	2.8	+	0
7	11.8	100	410	12.0	492	1.40	1	2.4	0	0
8	10.8	94	342	14.1	630	1.94	2	2.9	0	0
9	10.6	100	320	28.0	483	1.48	3	3.2	0	0
10	10.6	70	404	17.5	695	1.15	4	3.1	0	0
11	11.0	74	338	24.5	–	1.90	2	3.1	0	0
12	12.0	100	353	16.4	–	1.67	0	2.8	0	0
13	10.7	144	328	33.5	426	1.20	17	3.7	0	++
14	11.2	94	384	52.0	85	1.67	6	3.3	0	0
15	12.0	120	400	32.0	480	1.50	4	3.0	0	0
16	10.8	54	315	26.0	265	1.37	0	3.0	0	±
17	11.6	66	398	19.0	–	1.30	2	3.0	0	+
18	12.3	84	331	25.5	–	1.48	3	3.3	0	0
19	10.5	68	420	12.0	50	1.12	3	2.9	0	0
20	12.4	80	365	31.0	1030	1.53	1	3.1	0	±
21	12.4	90	375	36.8	820	1.40	6	3.1	0	0
22	11.7	58	350	32.0	–	0.90	15	3.7	0	±

TABLE III : CORRELATION BETWEEN "LOW" SERUM FOLATE AND INCIDENCE OF HYPERSEGMENTED NEUTROPHILS (5% OR MORE)

SERUM FOLATE LEVEL	INCIDENCE OF HYPERSEGMENTED NEUTROPHILS	
	ON ADMISSION	AFTER RECOVERY
LESS THAN 3 ng/ml.	2/5	0
MORE THAN 3 ng/ml.	4/16	8/22

Note : There is no correlation between the presence of hypersegmented neutrophils (5% or more) and the "low" serum folate level.

TABLE IV : CORRELATION BETWEEN SERUM IRON SATURATION AND INCIDENCE OF HYPERSEGMENTED NEUTROPHILS (5% OR MORE) DURING RECOVER FROM PCM

PATIENTS	SERUM IRON SATURATION		
	LESS THAN 15%	15–20%	MORE THAN 20%
WITH HYPERSEGMENTED NEUTROPHILS (8)	3	1	4
WITHOUT HYPERSEGMENTED NEUTROPHILS (14)	1	4	9

RESULTS

From the Tables I to IV as shows above it is evident that :

1. The presence of giant myeloid cells in the bone marrow and hypersegmented neutrophils (5% or more) is the common finding in children with severe protein calorie malnutrition especially before treatment.

2. There is no definite correlation between these findings and the blood levels of vitamin B_{12}, folates, vitamin E and iron. Thus, the interpretation of the presence of hypersegmented neutrophils and giant myeloid cells as the indication of megaloblastic dysplasia and or deficiency of above - mention hematinics in children with severe PCM may be misleading.

3. The patients who exhibit hypersegmented neutrophil more than 5% seem to have lower serum iron saturation than the others.

COMMENTS

The most constant leukocytic abnormality in pernicious anemia and other megaloblastic anemias is the presence of hypers gmented neutrophilic granulocyte (46) or Cooke's macropolycyte. (47) They may have 8, 10 or more nuclear lobes. (47,48) Their abnormal dimensions have been explained on the basis of pleurinuclearity. (49) Such cells are rarely seen in health but are found in the blood in the folic acid deficiency or pernicious anemia.

The changes in the neutrophilic polymorphonuclear leukocytes may be assets by either noting the variation in average lobe index or lobe value. (50-53) (ie the total number of nuclear lobes in in 100 neutrophils divided by 100) and by the "rule of fives" ie. noting the proportion of neutrophilic leukocytes having 5 or more nuclear lobes. Three percent being suggested as the upper limit for such cells in normal subject. (53) Herbert has used 3.17 ± 0.25 as the normal value but has suggested that the normal lobe average must be separately determined by each lab. (52) It is noted that the earliest hematological evidence of folate deficiency is an increase in "lobe average" (54,55) This increase in the lobe average is apparent 7 weeks after the onset of folate deprivation. Such hypersegmentation may be noted 2 weeks earlier in the bone marrow aspirates. (56) Herbert (55) stated that the lobe everage is of special value as a dianostic feature since it is not masked by concomittant iron deficiency but the other workers who studied pregnant women with folic acid and and iron deficiencies found that it may not be always true. (57) Tasker (58) had demonstrated that when iron depletion is the limiting factor in hemopoiesis the morphologic change in marrow and peripheral blood will be those of iron deficiency. When adequate amount of iron are supplied the morphological change may be then reflect the presence of other coexisting deficiencies such as that of folic acid (53, 57, 59)

Recently, the occurrence of gaint metamyelocytes and increased segmentation of polymorphonuclear leukocytes have been noted patients with iron deficiency anemia. (46, 53, 60-64) An experiment in the rats has demonstrated that iron deficiency led to a reduced fromiminotransferase activity and an increase in FIGLU excretion (65) similar to those observed in the iron deficient patients. (62) Arakawa et al (60) also described the patient with congenital formiminotransferase deficiency associated with neutrophilic hypersegmentation. Other studies also suggested the interrelation between the metabolism of iron and vitamin B_{12} (59), and iron and folates 65,67) It is postulated, therefore, that the iron

deficiency impaired the activity of the formiminotransferase enzyme and that this produce both the changes seen in neutrophils and the increased FIGLU excretion.

The other striking qualitative changes in leukocytes of the patients with megaloblastic anemias is the presence of the extraordinarily large leukocyte in the bone marrow and peripheral blood. This abnormal cellular development may occur at any state in the myeloid series but it is perticularly common among the metamyelocytes. It was thought that the macropolycytes are probably derived from these cells. (47) The frequent appearance of gaint neutrophilic band from and metamyelocytes with loose chromatin which is not typical of the Ehrlich megaloblast in children with PCM is also noted by many investigators. (7, 14, 19, 68-73, 76) Our patients as well as the other had their admission folate levels in the lower normal range or very low. (21,23-26,74,75) but the bone marrow picture were normal with occasional giant metamyelocytes which did not seem to have any correlation with serum folates, vitamin B_{12} or E. (23.76) As a matter of fact, we as well as the others found the elevated serum vitamin-B_{12} levels in PCM (19, 21, 23, 24, 26, 68,72,74,75,77) These high value have been blamed on (1) failure of tissue generally to take up vitamin B_{12} properly (2) failure of the liver specifically, because of injury, to retain vitamin B_{12} (78) Low serum vitamin E levels in severe PCM were noted by us and many others. (5,19, 23, 73, 74, 79-81) but its role in the pathogenesis of megaloblastic dysplasia in these children is still unsettled. (82)

The question whether the hypersegmented neutrophils noted in our patients are actually the polycytes of Ponder but not the classic macropolycyte was raised. The former have been observe in cases of infections but not related to megaloblastic anemias as do the latter. The hypersegmented neutrophil observed in our children were larger with less compact nuclear chromatin and more acidophilic cytoplasm similar to the classic macropolycytes. They were also found even when there is no evidence of infection or vitamin deficiency.

On admission, most of our childen had serum iron levels below the normal range comparable to the previous experiences (23,76) and supported the conclusion made earlier that iron deficiency is a conspicuous problem in PCM in Northern Thailand. (84) Serum iron levels are almost always low but not as low as, transferrin levels. This alteration produces high transferrin saturation in inspite of low aerum iron. (23,76,84-89) Whether the transferrin level is the most sensitive plasma marker for PCM remains to be seen. (85,90) Over the 12 weeks course there was a gradual drop in the stainable iron in the bone marrow even with adequate iron supplement. It is probably due to the impaired absorption from GI tract and markedly increased iron utilization during the hemoglobin regeneration.

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