

Original Research

Iron and Protein Sufficiency and Red Cell Indices in Phenylketonuria

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Objective and Methods: We reviewed records of 41 children with treated phenylketonuria (PKU) in order to evaluate hematopoiesis and the effect of iron and protein sufficiency.

Results: Six children (15%) were found to have anemia. Combined depletion of iron and protein stores was most likely to result in anemia, and two of the three children with this finding were anemic. Four children (10%) had evidence of iron deficiency without anemia (a precursor stage of iron deficiency anemia). Clinically significant iron depletion was found in older as well as younger children (well beyond the traditional infant/toddler deficient years). Plasma albumin was normal in all children and was not adequately sensitive to detect protein depletion sufficient to cause anemia or decreased growth. However, low plasma prealbumin (a more sensitive marker of protein sufficiency) correlated significantly with altered hematopoiesis or poor growth.

Conclusion: Compared to non-affected individuals, children with treated PKU make fewer red cells that have normal volume but increased hemoglobin per cell, resulting in a lower calculated hematocrit when measured by electronic cell counting. In the presence of iron or protein depletion, anemia may result. Routine monitoring of ferritin, complete blood counts and prealbumin are recommended for children with PKU at all ages.

INTRODUCTION

The dietary management of phenylketonuria (PKU) includes severe restriction of phenylalanine through the limitation of dietary protein and the addition of amino acids, vitamins and other nutrients through medical food (commonly known as “formula”). The loss of natural nutrients including heme iron, protein, vitamins and trace metals potentially affects hematopoiesis, and both iron and protein deficiency previously have been reported in PKU [1–6]. However, there is a paucity of information on the potential effects of the recommended PKU diet on red cell counts and indices in children with PKU. One prior study identified lower hemoglobin and higher mean corpuscular hemoglobin in children with treated PKU compared to children without PKU, although mean values were within the normal range [2]. Iron deficiency without anemia was apparent in several subjects in that study, but the role of protein sufficiency in hematopoiesis was not addressed. In contrast, another study did not identify any differences in

hematopoiesis [5]. We reviewed the hemoglobin, hematocrit and red cell count and indices in relationship to the iron and protein status of our patients with PKU to identify the extent to which iron or protein insufficiency might contribute to impaired hematopoiesis.

PATIENTS AND METHODS

We reviewed medical records of all children with classical PKU treated in the Inherited Metabolic Disorders Clinic at the University of Rochester School of Medicine and Dentistry/Children’s Hospital at Strong. Forty-one children consuming medical food and adhering to the prescribed phenylalanine restricted diet were studied. Several teens (from 15 to 18 years of age) were excluded for dietary non-compliance. All children were diagnosed by newborn screening and were continuously treated for PKU. Phenylalanine levels and diet records were reviewed every one to four weeks, depending on age and

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degree of compliance. Prealbumin and ferritin were performed at Strong Memorial Hospital as part of an annual clinic protocol for nutritional assessment. Ferritin deficiency was defined as ferritin $\leq 12 \mu\text{g/L}$, and marginal ferritin was defined as $\leq 20 \mu\text{g/L}$ [7]. Deficient prealbumin was defined as $< 15 \text{ mg/dL}$ and marginal prealbumin as $< 20 \text{ mg/dL}$ [8]. Blood counts and red cell indices were determined electronically by the Coulter counter (Coulter Diagnostics, Hialeah, FL), using the standard normal ranges for age and gender for this laboratory (Table 1). Children were sorted in five groups defined by age according to the normal ranges for red cell indices. The age groups were six months to two years (four children), two to six years (15 children), six to twelve years (15 children) and separate groups for males and females twelve years and older (three and four children respectively). A sufficient number of age- and gender-matched siblings were not available as a control group; thus, complete blood counts from two controls per subject were chosen from age- and gender-matched patients seen in the Emergency Department at Strong Memorial Hospital. Controls were screened to eliminate those with diagnoses that might affect red cell indices including blood loss, dehydration, acidosis or chronic illness. Patient and control samples were obtained by venipuncture. Ferritin or prealbumin controls were not available. Amino acids were analyzed by ion exchange chromatography on the Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, CA). In the study group, the amino acid analysis was obtained four to six hours post-prandial, concurrently with the other blood studies. The control group for amino acid analysis was chosen from age- and gender matched children undergoing amino acid analysis for developmental delay. These control samples were obtained four to six hours post-prandial in the same phlebotomy center and underwent the same specimen processing as study samples. Children with autism or who were on special diets were excluded as controls.

Compliance with medical food intake was assessed by patient/parent report and by reviewing frequency of requests for new medical food prescriptions. Dietary information consisting of reported medical food consumption, iron content of prescribed medical food and analysis of diet diary or three-day dietary recall was available for all patients. Children ages one to four were prescribed approximately 30 grams of protein per day as well as, on average, 145% of the United States Recommended Daily Allowance (RDA) for iron (in their medical food). This increased to 35 grams protein and 173% iron RDA for children four to seven, 40 grams protein and 158% iron RDA for ages seven to eleven, 50 grams protein and 114% iron RDA for females twelve and older and 55 grams protein and 111% iron RDA for males twelve and older.

Data were analyzed using Student's *t* test for difference of means, Pearson correlation coefficient and Chi-square including Fisher's exact test where appropriate.

Table 1. Subject vs. Control Means for Red Cell Indices

GROUP	n	HB (SD) (normal) (g/dL)	Control (SD)	p	HCT (SD) (normal) (%)	Control	p	RBC (SD) (normal) ($10^{12}/\text{L}$)	Control (SD)	p	MCH (SD) (normal) (pg)	Control (SD)	p
All Subjects	41	12.8 (0.9)	13.1 (0.2)	ns	36.6 (2.4)	38.2 (2.7)	0.002	4.4 (0.4)	4.6 (0.3)	0.001	29.3 (1.4)	28.2 (1.8)	0.001
6 mos-<2 yrs	4	11.9 (0.3) (11-14)	12.2 (0.8)	ns	34.5 (1.3) (34-40)	35.9 (2.2)	ns	4.2 (0.3) (3.9-5.5)	4.5 (0.2)	ns	28.5 (1.3) (23-31)	27.1 (1.4)	ns
2 yrs-<6 yrs	15	12.5 (0.6) (11.5-14)	12.6 (0.7)	ns	35.7 (1.9) (34-40)	37.3 (2.0)	0.016	4.3 (0.3) (3.7-5.3)	4.7 (0.3)	0.01	28.9 (1.4) (24-30)	27.3 (1.7)	0.01
6 yrs-<12 yrs	15	13.0 (0.8) (11.5-15.5)	13.1 (0.8)	ns	37.0 (2.2) (34-45)	38.4 (2.1)	ns	4.3 (0.3) (3.9-5.3)	4.6 (0.2)	0.05	29.7 (1.1) (25-33)	28.6 (1.8)	0.01
≥ 12 yrs-Boys	3	14.2 (0.6) (14-18)	14.9 (0.7)	ns	41.0 (2.0) (42-52)	42.3 (2.5)	ns	5.0 (0.6) (4.6-5.3)	5.0 (0.4)	ns	28.7 (1.2) (27-31)	29.7 (1.5)	ns
≥ 12 yrs-Girls	4	13.3 (0.6) (12-16)	13.8 (0.9)	ns	37.5 (1.9) (42-52)	40.9 (2.5)	0.05	4.4 (0.2) (4.2-5.4)	4.8 (0.2)	ns	30.8 (1.7) (27-31)	29.1 (1.5)	ns

HB = hemoglobin, HCT = hematocrit, RBC = erythrocytes, MCH = mean corpuscular hemoglobin.

RESULTS

The sample was composed of 41 children (24 boys and 17 girls) ranging in age from one year to 16 years. A complete blood count was available on all 41 patients. There was no significant difference in mean hemoglobin between the PKU group and the controls (Table 1). However, the hematocrit was significantly lower in the PKU group than in the control children, and the mean red cell count (RBC) was significantly lower in the PKU group than in the controls (Table 1). The mean corpuscular hemoglobin (MCH) was higher in the PKU group than the control (Table 1). Mean corpuscular volume (MCV) was within the normal range for all children, and there was no significant difference for the controls. Red cell distribution width (RDW) was within normal limits for all subjects. When the data were analyzed by individual age groups, the mean hemoglobin, hematocrit and RBC in the PKU group were below the control groups in all age ranges and the mean MCH was above the control range in all but the smallest sample size (boys ≥ 12 years). However, significance was found only in the age ranges with the largest sample sizes (Table 1).

Laboratory values for the study subjects with abnormalities of red cell indices or morphology, deficiency of ferritin or prealbumin or combined marginal ferritin and prealbumin are shown in Table 2. Six children of varying ages had hemoglobin or hematocrit below the lower limits of the laboratory's normal range (patients 1, 3, 5, 8–10). In three of these patients, this was associated with both low prealbumin and ferritin (patients 3 and 5) or with deficient prealbumin (patient 8). The other three children (patients 1, 9 and 10) had normal prealbumin and ferritin levels. Patient 9 subsequently had normalization of all red cell parameters after puberty.

In order to evaluate further any effect of iron sufficiency on red cell indices, we examined the ferritin levels of 28 patients. These patients' medical food prescriptions contained on average 156% of the RDA for iron (range 56% to 270%). One child had deficient ferritin, and nine children had marginal ferritin. The three year old child with deficient ferritin had apparent iron deficiency without anemia, as red cell numbers and size improved after additional ferrous sulfate administration (patient 4,

iron from medical food 200% of RDA). The nine children with marginal ferritin were from variable age groups. Two of the nine children were anemic, but these two also had low prealbumin levels (patients 3 and 5). After additional medical food and ferrous sulfate supplementation, both children had resolution of anemia. Patient 6 (with microcytosis determined by an examiner blinded to the study) moved from the area before ferritin was assayed or additional iron was supplemented.

Four PKU children consumed a diet that did not meet the US RDA for iron. These included two teen girls, one teen boy and one ten year-old boy. Two of these children had marginal ferritin levels. None in this group had frank anemia, but three of the four children (all but the teen boy) had hemoglobin and hematocrit below the mean for their age group.

The difference in mean ferritin levels between children less than age six (27.4 ng/mL) and children who were age six or older (38.7 ng/mL) was not significant ($p = 0.08$). In order to assess if low ferritin might simply be a marker of noncompliance with medical formula intake or protein deficiency, we looked for a correlation between ferritin and prealbumin, but none was found ($r = -0.16$, $p = 0.41$).

It was apparent that the decrement in hematocrit in our PKU patients could not be solely attributed to low iron stores. We also studied protein sufficiency to determine its relationship to hematopoiesis. Albumin and total protein levels were reviewed and were normal in all 41 patients, and the group means and distributions for these parameters did not differ significantly from the normal ranges. However, prealbumin levels, also available on all 41 patients, were frequently low. Prealbumin was lower in children less than six years of age when compared to those older than six (19.5 vs. 22.1 mg/dL, $p = 0.03$). There was also a significant correlation between prealbumin and phenylalanine level such that children with lower phenylalanine levels also had lower prealbumin ($r = 0.38$, $p = 0.02$). Sample size was not sufficient for multivariate statistics to separate the effects of age vs. prealbumin on hemoglobin.

Prealbumin deficiency was found in two children, both poorly compliant with medical food consumption (patients 7 and 8). Patient 8 had normalization of hemoglobin, hematocrit, and prealbumin and improvement in short stature following an

Table 2. Individual Values in Subjects with Abnormalities in Red Cell Indices, Deficiency of Ferritin or Prealbumin, or Combined Marginal Prealbumin and Ferritin

Subject	Age-Gender	Current Phe ($\mu\text{mol/L}$)	Hb (g/dL) (normal)	Hct (%) (normal)	MCV (fL) (normal)	Morphology	Ferritin (ng/mL)	Prealbumin (mg/dL)	Albumin (g/L)
1	22 mos-Male	1087	11.7 (11–14)	33 (34–40)	84 (71–84)	normal	26	20	41
2	21 mos-Male	174	12.3 (11–14)	36 (34–40)	80 (71–84)	normal	20	18	41
3	34 mos-Male	217	11.2 (11.5–14)	32 (34–40)	81 (76–87)	normal	20	19	44
4	3 yrs-Male	60	12.3 (11.5–14)	36 (34–40)	78 (76–87)	microcytosis	11	22	39
5	4 yrs-Female	374	11.5 (11.5–14)	33 (34–40)	86 (76–87)	normal	19	19	43
6	4 yrs-Female	236	12.3 (11.5–14)	34 (34–40)	78 (76–87)	microcytosis	NA	23	45
7	7 yrs-Female	767	13.3 (11.5–15.5)	38 (35–45)	83 (77–85)	normal	58	14	48
8	8 yrs-Male	381	11.1 (11.5–15.5)	32 (35–45)	79 (77–85)	normal	75	12	37
9	13 yrs-Male	36	13.6 (14–18)	39 (42–52)	80 (80–87)	normal	29	21	41
10	14 yrs-Male	260	14.3 (14–18)	41 (42–52)	80 (80–87)	normal	27	23	46

increase in medical food consumption. Patient 7 had normal red cell indices, but still had a modest increase in hemoglobin and hematocrit following an increase in medical food consumption. Marginal prealbumin was found in twelve children. These patients spanned all age ranges and were among our most compliant patients both with respect to maintenance of phenylalanine levels and consistent medical food consumption. Three of these patient also had marginal ferritin levels, and in addition two of these three patients were anemic (patients 3 and 5 discussed previously).

Plasma amino acid analysis was available on 39 patients. One patient had a deficiency of three amino acids (threonine, leucine, tyrosine), and eight children had a deficiency of one essential amino acid, including tyrosine (3), leucine (1), threonine (1), valine (1), isoleucine (1), methionine (1). These results were not confined to a particular age group and were found to be distributed over all ages. The control group had significantly fewer children with an essential amino acid deficiency (9 PKU children vs. 1 control child, $p = 0.01$). The PKU children with low prealbumin were more likely to have an essential amino acid deficiency ($p = 0.05$). Asparagine deficiency was present in twelve patients, but only in two controls ($p = 0.01$). Variable age distribution of medical food choice prevented a comparison of iron or protein sufficiency by medical food (Table 3).

DISCUSSION

This paper confirms the prior study of Beatriz *et al.* [2] both in regard to the presence of altered hematopoiesis in children with PKU and the relatively common identification of iron deficiency without anemia in these children. In addition, we have identified a previously unreported association between protein insufficiency and impaired hematopoiesis. The combination of iron and protein insufficiencies appears to have a more pronounced effect in impairing red cell production than just one insufficiency alone. Overall, children with PKU undergoing dietary treatment make fewer red cells (RBC) which contain a slightly increased amount of hemoglobin per cell (MCH). This results in greater preservation of total hemoglobin than hematocrit. Because hematocrit is a derived value on the Coulter Counter (the product of RBC and MCV), the lower

hematocrit values in our patients actually reflect the decreased red cell count. Since red cell indices generally fall within the normal range, the clinical significance of the hematopoietic changes in otherwise healthy, nutritionally replete children is small in the aggregate, but in some cases results in anemia.

Decreased iron stores in children with treated PKU in spite of adequate iron intake has been reported previously and has been attributed to decreased bioavailability of iron obtained from vegetable and synthetic sources [1–5]. Typically in non-PKU children iron deficiency is a disorder of early childhood. However, we found children with PKU may have reduced iron stores at any age, and thus they are at increased risk for true iron deficiency well beyond the traditional screening years.

Classic iron deficiency is associated with microcytic anemia and irreversible cognitive impairment [9–11]. The milder condition known as iron deficiency without anemia typically presents with normochromic, normocytic red cells and low hemoglobin and hematocrit values which remain within normal limits for age but increase with iron supplementation [12,13]. Although none of our patients had frank anemia based on iron deficiency alone, several had iron deficiency without anemia (patients 3, 4 and 5 and also most likely patient 6, who moved from the area prior to further evaluation). Thus, decreased ferritin stores may explain some of the overall depression of hematocrit in our patients compared to the controls even though our patients' indices were generally within the normal range.

Low prealbumin levels in children with PKU have been reported previously [1,6], and protein inadequacy has been proposed to cause decreased growth and bone density [14–18]. However, many PKU treatment centers do not routinely follow prealbumin levels in their patients. Prealbumin (transthyretin) is a much more sensitive predictor of plasma protein status than albumin [19–21], particularly because albumin levels are preferentially preserved during mild protein depletion (through a reduction in albumin degradation) when total calories remain adequate [19]. Low prealbumin in the presence of normal albumin levels might also be caused by a brief period of starvation prior to the clinic visit (possibly in an attempt to lower plasma phenylalanine levels). Since prealbumin levels were lowest in the youngest children, this was unlikely to have been a major confounder. The lower prealbumin levels in the younger children in our study most likely reflect the higher growth rate and amino acid utilization in this age group. Protein

Table 3. Medical Food Choice, Amino Acid Deficiencies, Iron Content and Age-Specific Distribution

Medical Food	n	Essential Amino Acid Deficiency	Asparagine Deficiency	Iron Prescribed (% RDA)	6 mos–<2 yrs	2 yrs–<6 yrs	6 yrs–<12 yrs	≥12 yrs Male	≥12 yrs Female
Phenylfree ^a	22	5	8	179%	1	7	12	1	1
Periflex ^b	11	3	1	151%	2	7	0	1	1
PKU-2 ^c	4	1	2	78%	0	0	1	1	2
Other	4	0	0	NA	1	1	2	0	0

^a Mead Johnson, Evansville, Indiana.

^b Scientific Hospital Supplies North America, Gaithersburg, Maryland.

^c Milupa, Friedrichsdorf, Germany.

sufficiency is also affected by the characteristics of timing and absorption of L-amino-acid-containing medical foods [22–26] and by poor compliance with medical food consumption [27]. The extent to which noncompliance, poor absorption of L-amino acids or timing of medical food ingestion contributed to low prealbumin in our patients is not known. The potential relationship we identified between hemoglobin and prealbumin values merits further study to separate the effects of age.

The specific standards for marginal or deficient ferritin or prealbumin values in normal children vary somewhat between references. In our study, the lowest ferritin value (11 ng/dL) in the presence of sufficient prealbumin was associated with iron deficiency without anemia; however, in some cases higher ferritin levels (as high as 20 ng/dL) were associated with abnormal hematopoiesis if prealbumin was also marginal or deficient. Disturbances in growth or hematopoiesis were also found when the prealbumin was at or below 14 mg/dL; levels up to 19 mg/dL were also associated with altered hematopoiesis if iron stores were marginal as well. Thus, it appears the more stringent criteria for iron or protein sufficiency of Dienard [7] or Inglebleek [8] may be more appropriate goals for children with PKU. Our data also suggest that prealbumin is superior to albumin for routine nutritional monitoring in PKU.

Other nutritional deficiencies that may contribute to impaired hematopoiesis include micronutrients, vitamins, fatty acids and others. Though these particular deficiencies were not completely assessed in this study, both selenium and B-12 deficiency have been described in children with PKU [28–31]. Patient 3 in our study had zinc as well as ferritin deficiency (zinc 64 $\mu\text{g/dL}$, normal 70–140) and patient 7 had normal selenium. We cannot rule out a broader or combined effect of multiple nutritional deficiencies contributing to the altered hematopoiesis in our patients.

Strict control of blood phenylalanine level may result in increased reliance on medical foods for nutritional adequacy. Our data suggest regular monitoring of complete blood counts and ferritin may be indicated regardless of age in order to detect underlying nutritional deficiencies and preserve normal hematopoiesis and growth. Routine assessment of plasma prealbumin, rather than albumin, is also recommended to monitor protein sufficiency. Additional studies of children with other disorders requiring protein restriction or elemental diets may provide information concerning whether the altered hematopoiesis is unique to PKU or if it represents a more general response to dietary restriction of natural protein, heme iron and other nutrients.

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