IRON STATUS OF FILIPINO INFANTS AND PRESCHOOLERS USING PLASMA FERRITIN AND TRANSFERRIN RECEPTOR LEVELS

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Abstract. Iron status of 1,861 Filipino infants and preschoolers was evaluated by measurements of plasma ferritin (PF), transferrin receptor (TR) and hemoglobin (Hb). One group of subjects (Group I) consisted of all anemic subjects together with a systematic subsample from the Fourth National Nutrition Survey-Biochemical Phase. Results showed that depleted iron stores based on PF (< 12.0 ng/ml) was present in 70.0% of infants and 60.5% of preschoolers. Tissue iron deficiency based on TR (> 8.5 mg/l) was present in higher proportion (80.0% and 73.7% for infants and preschoolers) which was comparable to the proportion of anemia (80.3%). In a subgroup of subjects from the Country Program for Children IV (Group 2) elevated TR was present in 61.4% of infants and 46.5% of preschoolers. A lower proportion of depleted iron stores of 22.7% in infants and 15.2% in preschoolers was observed. Correlation test showed that there was a closer relationship between Hb and TR (r = -0.42) than Hb and PF (r = 0.20) even if PF was expected to give a higher proportion of values below normal. The occurrence of anemia in the presence of elevated TR without any decrease in PF values suggest that the diagnostic ability of PF could be limited in the presence of infection. Therefore, future studies should include biochemical tests such as C-reactive proteins (CRP) to determine the extent of association between anemia and infection.

INTRODUCTION

The fourth national nutrition survey (1V NNS) conducted in 1993 showed a prevalence of anemia of 49.2% in infants and 26.7% in children aged one to six years (Kuizon *et al*, 1994). However, not all cases of anemia would be due to iron deficiency nor would all cases of iron deficiency be severe enough to cause anemia. Other nutrients necessary for hematopoiesis may also be deficient in the diet, including folic acid, vitamin A, vitamin C, riboflavin, protein, copper and other minerals. Common infections, especially chronic and recurrent ones, cause some impairment of hematopoiesis and consequent anemia. Some parasitic infections notably malaria cause additional blood loss from hemolysis. Iron deficiency anemia represents the more severe form of iron deficiency, but there are also milder forms of iron deficiency where anemia is absent (WHO/UNICEF/UNU, 1994). Thus, some additional laboratory assessments in addition to hemoglobin determination is desirable to identify iron lack as the cause of the anemia.

A number of well-established tests other than hemoglobin (Hb) can be used in assessing iron status. Earlier studies on nutritional anemia in Filipinos were based on Hb and transferrin saturation (Marzan, 1970, Kuizon *et al*, 1979). Later studies included erythrocyte protoporphyrin (EP) and serum ferritin (SF) (Kuizon *et al*, 1982, 1989). The use of these iron parameters, however, is problematic in areas where infections are common. A SF in the normal range can not always be taken to indicate iron sufficiency without some certainty that the individual is free from an infectious or inflammatory condition. Infection or inflammation, lead poisoning and hemolytic anemia can cause significant elevation of EP (WHO/UNICEF/UNU, 1994).

The measurement of serum transferrin receptor (TR) has been reported as one of the most important developments in assessing iron status (Trowbridge, 1989). This parameter evolved from studies of the mechanism by which iron is transported from circulation to the intracellular compartment (Cook, 1989). The receptor binds diferric transferrin. Since the rate of receptor synthesis is linked to the iron needs of the cell, serum receptor levels rise significantly with tissue iron deficiency to allow the cells to compete effectively for diferric transferrin (Carriaga *et al*, 1991). One major advantage of TR is the fact that it is not significantly affected by infection or the inflammatory process (Ferguson *et al*, 1992) and thus may be the preferred tool for settings where infections are common. Thus, combined SF and TR measurements will assist in identifying the cause of anemia detected in population surveys (Cook *et al*, 1993).

This report presents the results of a case study to determine the proportion of iron deficient subjects in a group of Filipino infants and children using plasma ferritin (PF) and TR.

MATERIALS AND METHODS

A sub-sample from the subjects of the biochemical phase of the 1V NNS aged six months to six years (Kuizon *et al*, 1994) was taken and designated as Group 1. Sub-sampling was done by including all subjects with Hb below normal (WHO, 1972) and a 20% systematic sample from those with normal levels (Group 1a). PF was analysed with the ELISA method using monoclonal reagents (Flowers *et al*, 1986). Some remaining samples with sufficient volume (Group 1b) were analysed for TR (Flowers *et al*, 1989). Both analyses were done at Kansas University Medical Center (KUMC), Kansas City, USA, in collaboration with Dr Cook. Results from Group 1, which showed elevated TR in subjects with normal PF led to another investigation where more samples were analysed for PF and TR. All samples with sufficient volume for PF and TR assays from subjects of the Country Program for Children (CPC) 1V were designated as Group 2 subjects. These were brought to KUMC for analysis of TR, while PF was analyzed at the Food and Nutrition Research Institute using SF standards and reagents supplied by KUMC in collaboration with Dr Cook.

Anthropometric data on both groups of subjects were requested from the Nutrition Assessment and Monitoring Division and included in the evaluation of results to determine the contribution of malnutrition to the anemia problem. One month history of infection (upper respiratory tract infection (URTI), respiratory tract infection (RTI), measles and diarrhea) which were recorded by the biochemical researchers during the survey, was also included to help in the proper interpretation of findings.

Malaria, which could cause anemia through hemolysis and depressed marrow response, was also considered. Iron parameters were compared in subjects from provinces which are endemic and non-endemic for malaria (DOH, 1991).

Statistical analysis

Logarithmic transformation was done for PF data. Generation of data (mean and standard deviation, prevalence of deficiency, *t*-tests) were done using the Statistical Package for the Social Sciences (SPSS) program.

RESULTS

Proportion of anemia and iron deficiency

The proportion of Hb below normal in Group 1a was 82.6% in 138 infants and 67.4% in 945 preschoolers aged 1 to 6 years. On the other hand, much lower proportions of 24.6% of infants and 24.0% ($p < 0.01$) of preschoolers were iron-depleted as indicated by PF < 12 ng/ml (Table 1).

Shown also in Table 1 are results on 162 subjects with values on PF and TR (Group 1b). Depleted iron stores were indicated in 70.0% of infants and 60.5% of preschoolers based on PF. Using TR > 8.5 mg/l (WHO/UNICEF/UNU, 1994), tissue iron deficiency was present in a higher proportion of 73.7% ($p < 0.05$) of preschoolers. This value was comparable to the proportion of anemia of 80.3%.

Based on abnormalities in both PF and TR as shown in Table 2, 6 out of 10 infants (60.0%) and 80 out of 152 preschoolers (52.6%) were iron deficient (ID). Iron deficiency anemia (IDA) was present in 60.0% also of infants and 77 out of 152 preschoolers (50.7%) because of concurrent anemia in addition to abnormal values of PF and TR.

It is noteworthy that out of 122 anemic preschoolers, 13 had normal PF (12-50 ng/ml), 5 had normal PF (50.1-100 ng/ml) and 3 had highly elevated PF (> 100 ng/ml) even when the TR was already elevated (> 8.5 mg/l) as shown in Table 2. Iron deficiency can be ruled out in these cases (Ferguson *et al*, 1992).

Table 1

Hemoglobin, plasma ferritin and transferrin receptor levels in infants and children.

	Group 1a		Group 1b		Group 2	
	6 mo- < 1 y (138)	1 - 6 y (945)	6 mo- < 1 y (10)	1 - 6 y (152)	6 mo - < 1 y (44)	1 - 7 y (572)
Hemoglobin (g/dl)						
X ± SD	10.2 ± 1.1	10.8 ± 1.5	10.1 ± 1.7	10.2 ± 1.4	10.4 ± 1.6	11.6 ± 1.4
% below normal[1]	82.6	67.4	80.0	80.3	59.1	32.0
Plasma ferritin (ng/ml)						
GM	20.8	23.7	9.5	11.7	23.4	30.2
% below normal[2]	(7.9 - 54.5)	(7.8 - 71.6)	(7.0 - 12.0)	(8.1 - 15.3)	(19.3 - 24.3)	(27.7 - 32.4)
	24.6	24.0	70.0	60.5	22.7	15.2
Plasma transferrin receptor (mg/l)						
X ± SD			14.3 ± 5.3	13.8 ± 6.8	10.4 ± 4.7	9.2 ± 4.1
% elevated[3]			80.8	73.7	61.4	46.5

[1]Hb < 11 g/dl 6 months to 6 years (WHO, 1972)
< 12 g/dl 6 to 14 years
[2]PF < 12 ng/ml (WHO/UNICEF/UNU, 1994)
[3]TR > 8.5 mg/l (WHO/UNICEF/UNU, 1994)

Table 2

Number of anemic and non-anemic subjects distributed according to PF and TR (Group 1).

PF ng/ml	Iron depleted < 12.0		Normal 12.0 - 50.0		Normal 50.1 - 100.0		Highly elevated > 100.0	
TR (mg/l)	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5
Infants								
Anemic (8)	6	1	0	0	1	-	-	-
Non-anemic (2)	-	-	1	1	0	-	-	-
Total (10)	6	1	1	1	1	-	-	-
Pre-schoolers								
Anemic (122)	77	11	13	4	5	7	3	2
Non-anemic (30)	3	1	7	8	4	7	-	-
Total (152)	80	12	20	12	9	14	3	2

Anemic (WHO, 1972)
6 months to 6 years Hb < 11.0 g/dl
6 to 14 years < 12.0 g/dl

Group 2

Anemia was indicated in 59.1% of infants and 32.0% of preschoolers, while tissue iron deficiency based on TR was indicated in 61.4% and 46.5%, respectively. In contrast, lower proportions ($p < 0.01$) of 22.7% and 15.2%, respectively, had depleted iron stores based on PF as shown also in Table 1. Using PF, TR and Hb, the proportion of abnormal values were all higher in infants than in preschoolers.

Table 3 shows that 19 out of 44 infants (43.2%) had below normal Hb and elevated TR while only 6 out of 44 (13.6%) and below normal Hb as well as PF. Using abnormalities (PF < 12 ng/ml and TR > 8.5 mg/l) in both PF and TR, only four out of 44 (9.1%) had IDA. Similarly, in preschoolers a higher proportion of 133 out of 572 (23.3%) had elevated TR and below normal Hb compared to 49 out of 572 (8.6%) with below normal Hb and PF. TR was elevated in 10 subjects who had highly elevated PF (> 100 ng/ml).

Correlation between iron parameters in Group 2

The correlation between log PF and Hb ($r = 0.23$, $p < 0.001$) was lower than the correlation between TR and Hb ($r = -0.40$, $p < 0.001$). A much lower correlation ($r = 0.17$, $p < 0.001$) was obtained between TR and log PF as shown in Table 4. Fig 1, 2 and 3 show the relationship between the iron parameters in Group 2. The lack of close correlation between the iron parameters is due to the fact, that each test reflects different aspects of iron metabolism (Dallman *et al*, 1981).

Table 3

Number of anemic and non-anemic subjects distributed according to PF and TR (Group 2).

PF ng/ml	Iron depleted < 12.0		Normal 12.0 - 50.0		Normal 50.1 - 100.0		Highly elevated > 100.0	
TR (mg/l)	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5	Elevated > 8.5	Normal ≤ 8.5
Infants								
Anemic (26)	4	2	10	5	3	-	2	-
Non-anemic (18)	3	1	4	8	1	1	-	-
Total (44)	7	3	14	13	4	1	2	0
Children								
Anemic (183)	44	5	66	36	13	6	10	3
Non-anemic (389)	13	25	87	135	23	60	10	36
Total (572)	57	30	153	171	36	66	20	39

Anemic (WHO, 1972)
6 months to 6 years Hb < 11.0 g/dl
6 to 14 years < 12.0 g/dl

Table 4

Correlations between iron parameters in 615 subjects (Group 2).

	Log PF	TR	Hb
Log PF	1.000	- 0.1746*	0.2324*
TR	- 0.1746*	1.000	- 0.4023*
Hb	0.2323*	- 0.4023*	1.0000

* $p < 0.001$

Anemia and malnutrition

Table 5 shows that the proportion of anemic children tended to be higher in wasted children (41.0%) than in normal children (33.0%). The proportion of depleted iron stores (PF < 12 ng/ml) tended to be even lower in wasted children (11.5%)

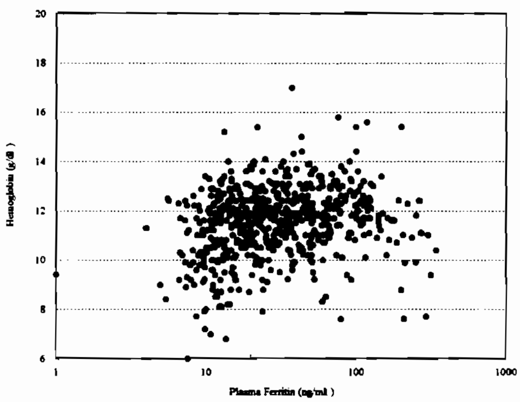

Fig 1–Association between plasma ferritin and hemoglobin in infants and children (6 months to 7 years).

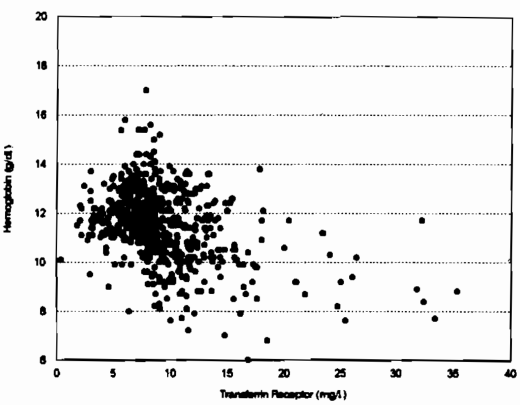

Fig 2–Association between TR and Hb in infants and children (6 months to 7 years).

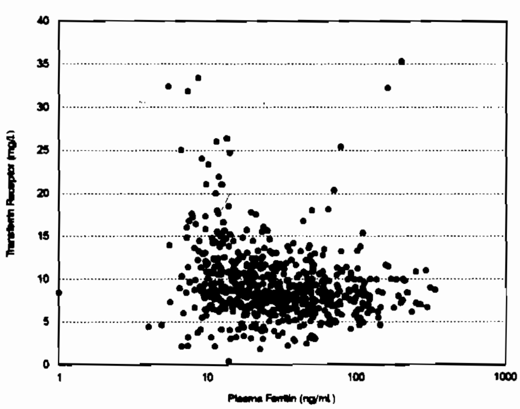

Fig 3–Association between TR and ferritin in infants and children (6 months to 7 years).

than in normal children (16.6%). The proportion of elevated TR values did not differ in normal (48.6%) and in wasted children (47.5%). However, the big difference in sample size between normal (518) and wasted children (61) was a limitation in the above comparison.

Anemia and infection

Table 6 shows that anemia was present in a higher proportion ($p < 0.05$) of 34.3% of those with one month history of infection (Group A) compared with 26.8% of those without history of recent infection (Group B). The proportion of subjects with below normal PF did not differ in Group A (16.6%) and those in Group B (15.5%). Furthermore, 74.3% of PF levels were within the range of 12-100 ng/ml. The proportion of normal and elevated TR did not differ (50.3 *vs* 49.7) in Group A.

There was no consistent trend in proportion of elevated TR in provinces reported as endemic for malaria compared to non-endemic provinces such as Aklan, Capiz and Northern Leyte and National Capital Region (data not shown).

DISCUSSION

The PF was expected to give a higher proportion of below normal values because it depends on an indicator of early reduction in iron stores. The failure of PF to yield higher proportion of iron depletion than TR may reflect increasing influence of factors other than iron deficiency. Elevated PF concentrations were reported in children with mild infections such as upper respiratory infections (Reeves *et al*, 1984). PF values are commonly within the normal range of 20-100 µg/l when infection and iron deficiency co-exist (Cook, 1989).

Multiple iron biochemistry tests (*eg* MCV model has mean corpuscular volume (MCV), transferrin saturation (SAT) and EP, and Ferritin model has SF, SAT and EP) had been used in the National Health and Nutrition Examination Surveys (NHANES; ESWG, 1985) to obtain a precise assessment of iron status (Cook *et al*, 1976). On the other hand, Hallberg *et al* (1993) reported that the use of several parameters results in underestimation of iron deficiency. Their finding was supported by the results of the present study. For example in Group 2, whereas 43.2% of the infants could be classified as IDA based on TR and Hb,

Table 5

Distribution of different iron parameters according to types of malnutrition[1] (Group 2).

Nutritional status	No.	Hb Percent distribution		PF Percent distribution				TR Percent distribution	
		Anemic	Non-anemic	Depleted	Normal		Elevated	Normal	Elevated
				< 12.0	12 - 50	50.1 - 100	> 100	≤ 8.5	> 8.5
Normal	518	33.0	67.0	16.6	56.9	16.2	10.2	51.4	48.6
Malnourished									
Wasted	61	41.0	59.0	11.5	60.7	18.0	9.8	52.5	47.5
Stunted	33	36.4	63.6	18.2	48.5	24.2	9.1	66.7	33.3
Both wasted and stunted	3	33.3	66.7	0.0	33.3	66.7	0.0	33.3	66.7

[1] Normal - > P5 of standard weight-for-height and > P5 standard height-for-age
Stunted only- > P5 of standard weight-for-height and ≤ P5 of standard height-for-age
Wasted only - ≤ P5 of standard weight-for-height and > P5 of standard height-for-age
Both Wasted and stunted - ≤ P5 of standard weight-for-height and ≤ standard height-for-age

Table 6

Percentage distribution of Hb, PF and TR in subjects with and without history of infection (Group 2).

	Hb Percent distribution		PF Percent distribution				TR Percent distribution	
	Anemic	Non-anemic	Depleted	Normal		Highly elevated	Normal	Elevated
			< 12	12 - 50	50.1 - 100	> 100	≤ 8.5	> 8.5
Group A								
With history of recent infection (338)	34.3*	67.7	16.6	58.6	15.7	9.1	50.3	49.7
Group B								
Without history of recent infection (277)	26.6	73.4	15.5	54.5	18.8	11.2	55.7	44.3

( ) no of subjects
* $p < 0.05$

only 9.1% could be classified as IDA based on below normal Hb in addition to abnormalities in both PF and TR. If conclusions were based on Hb and TR only because of the limitations of PF in the Philippines, where iron deficiency states are often complicated by infections, 43.2% of the infants and 23.3% of the preschoolers could be classified as IDA. Since 43.2% is nearly 50%, it might be possible that some of the remaining children have some degree of iron deficiency but not severe enough to cause anemia (FAO/WHO/UNICEF, 1994). This finding supports the DOH comprehensive nutrition program wherein ferrous sulfate drops (15 mg elemental iron daily for 2 months) are distributed and administered to all infants aged 6 to 11 months.

There is no single test that can diagnose iron deficiency. Low serum ferritin values provide unequivocal evidence of iron deficiency, but a normal to high value may indicate either adequate iron stores or infection. Transferrin receptor in the absence of increased erythropoiesis maybe a better tool for iron assessment, since this is not affected by infection. In areas where infection and other tropical diseases are common, evaluation of iron status should include serum ferritin, transferrin receptor and hemoglobin. The simultaneous use of these measurements will not only establish the true prevalence of iron deficiency but also measure its severity.

To show infection as a probable factor contributing to high rates of anemia, a more comprehensive study should be done including measures of infection such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) or plasma viscosity test. Although, these indices give nonspecific diagnostic values, they are useful for the differential diagnosis of iron deficiency and chronic diseases (Johnson, 1989). Blood smear for malarial parasites should also be examined especially in subjects living in endemic areas since malarial infection may elevate serum transferrin receptor due to increased red cell hemolysis (Ferguson *et al*, 1992).

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