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Multimicronutrient Biomarkers Are Related to Anemia during Infancy in Indonesia: A Repeated Cross-Sectional Study

Aly Diana [1,2] Dwi M Purnamasari,[1] Sofa Rahmannia [1] Dimas E Luftimas,[1] Jillian J Haszard [2] Rosalind S Gibson,[2] and Lisa A Houghton[2]

[1]Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia and [2]Department of Human Nutrition, University of Otago, Dunedin, New Zealand

ABSTRACT

Background: Anemia during infancy in Indonesia is common, with iron deficiency (ID) assumed to be the major cause. Other micronutrients besides iron may have a role in determining hemoglobin (Hb) but have not yet been explored in Indonesia.

Objective: We investigated 7 micronutrient biomarkers and selected nonnutritional factors as potential predictors of Hb and anemia at ages 6, 9, and 12 mo in a cohort of Indonesian infants at risk of coexisting micronutrient deficiencies.

Methods: Apparently healthy breastfed infants were randomly selected from birth registries at 6 mo ($n = 230$) and followed-up at 9 mo ($n = 202$) and 12 mo ($n = 190$). Hb, serum micronutrient biomarkers—iron [as ferritin and serum soluble transferrin receptor (sTfR)], zinc, selenium, folate, vitamin A [as retinol-binding protein (RBP)], vitamin B-12, and vitamin D (as 25-hydroxyvitamin D) (adjusted for inflammation, where appropriate)—and maternal sociodemographic status, health, BMI, heminthiasis, and selected Hb genetic disorders were measured. Multivariate analysis examined relations between micronutrient biomarkers and nonnutritional factors (except helminthiasis and genetic Hb disorders) with Hb and anemia at 6 and 12 mo.

Results: ID (based on ferritin) was a predictor of lower Hb and anemia at both 6 and 12 mo of age ($P < 0.02$). Additional predictors at 6 mo were tertiary education and higher maternal Hb for higher Hb, sex (being male) and inflammation ($P < 0.05$) for both lower Hb and anemia, and greater maternal height ($P = 0.036$) for anemia only. At 12 mo, a significant biomarker predictor besides ID was RBP ($P = 0.035$) for Hb.

Conclusion: ID was a major contributor to lower Hb and anemia, although RBP was also associated. *Curr Dev Nutr* 2019;3:nzz022.

Keywords: anemia, hemoglobin, Indonesia, infants, iron, micronutrient biomarkers, retinol-binding protein

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

Address correspondence to AD (e-mail: diana.aly@gmail.com).

Abbreviations used: AGP, α 1-acid glycoprotein; APP, acute phase protein; APR, acute phase response; CRP, C-reactive protein; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; RBP, retinol-binding protein; sTfR, serum soluble transferrin receptor.

Introduction

Anemia is a widespread problem in many low- and middle-income countries, and remains persistent during infancy and early childhood in Indonesia. Almost 30% of preschool children in Indonesia are anemic according to the latest national data (1), with rates even higher during infancy depending on age and geographic region (2–4). Despite limited data, iron deficiency (ID) has been assumed to be the major cause of anemia in Indonesia (5).

Increasingly, the contribution of many other coexisting factors besides ID to the overall burden of anemia in childhood is being recognized. These include multiple micronutrient deficiencies, parasitic infections, inflammation, and in some regions genetic hemoglobin (Hb) disorders (6, 7). However, to date their relative importance as predictors of Hb during infancy in Indonesia has not been investigated.

In addition to iron, other micronutrients such as folate, vitamin B-12, and vitamin A have well-established roles in normal hematopoiesis (8). However, emerging evidence suggests that zinc, selenium, and vitamin D may also be involved through several plausible mechanisms (6, 9). Earlier studies in Indonesia have documented deficiencies of iron, vitamin A, and zinc during infancy (2, 10, 11) but, to our knowledge, there are limited data for the other micronutrients.

In disadvantaged settings, when infants begin to crawl around the home and eat solid foods, they may become exposed to infective eggs of soil-transmitted helminths (12) which, depending on the intensity of infection, have the potential to induce anemia through both blood loss (13) and malabsorption of micronutrients (14). Losses of micronutrients such as iron, zinc, vitamin A, and selenium can compromise the immune system, thus increasing the susceptibility of the infants to infections and inflammation. During inflammation, inflammatory cytokines initiate the acute phase response (APR), during which there are alterations in the concentrations of numerous plasma proteins regulated primarily by the liver. Protein concentrations that are increased or decreased in the plasma during the APR are classified as positive or negative acute phase proteins (APPs), respectively, and include ferritin (positive APP) and retinol-binding protein (RBP) (negative APP). As a consequence, micronutrient biomarker concentrations such as plasma ferritin are temporarily elevated, whereas others such as serum RBP are temporarily reduced during the APR, even though there is no change in micronutrient status. This leads to inaccurate assessments of micronutrient status and over- or underestimates of the prevalence of deficiency in a population during inflammation (15). In addition, both absorption of iron and the release of iron from body stores are reduced in response to inflammation, and as a consequence functional ID may develop and, ultimately, iron deficiency anemia (IDA) (16).

In many Southeast Asian countries, hereditary disorders affecting the structure, function, and/or production of Hb are widespread. Data on genetic Hb disorders in Indonesia are limited, although β-thalassemia, Hb E, and α-thalassemia have been reported, with ranges of carrier frequency rates of 5–10%, 1–33%, and 6–16%, respectively, depending on ethnicity (17). Some of these genetic Hb disorders present as mild to severe anemia (18, 19), although the extent to which they contribute to low Hb concentrations in Indonesia in later infancy remains uncertain.

We previously reported on the adequacy of both complementary feeding patterns (20) in a longitudinal study of infants from 6 to 12 mo of age living in Sumedang district, West Java, Indonesia. In addition, we applied the new Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia approach (21) to adjust 4 micronutrient biomarkers of iron, zinc, vitamin A, and selenium for inflammation (22). In this study, we extend our research to investigate the role of 3 additional micronutrient biomarkers—folate, vitamin B-12, and vitamin D—together with the 4 micronutrient biomarkers studied earlier (22), as well as selected nonnutritional factors as potential predictors of Hb and anemia among these Indonesian infants. We hypothesized that low status of 6 micronutrients (folate, vitamin B-12, vitamin A, zinc, selenium, and vitamin D), in addition to iron, would be independently associated with Hb.

Methods

Study design and participant selection

Details of the study design, selection and characteristics of the participants, and justification for the sample size based on the prevalence of stunting were described earlier (20, 22). Briefly, apparently healthy breastfed infants randomly selected from local birth registry data were recruited at age 6 mo (n = 230) from 30 villages in 3 subdistricts of Sumedang district, West Java, and followed-up at ages 9 mo (n = 202) and 12 mo (n = 190) from August, 2013 to August, 2014. Ethical approval of the study protocol was obtained from the Human Ethics Committees of University of Otago, New Zealand (H14/022) and the Universitas Padjadjaran, Indonesia (No 132/UN6C2.1.2/KEPK/PN/2014). Parents of the infants provided informed written consent for the study. The respondents were compensated for their transportation costs for the health clinic visits, and provided with the results from the anthropometric assessment of growth, complete blood count, and the blood group of their infants.

After recruitment, pretested questionnaires were used to collect information on sociodemographic status, health, infant and young child feeding practices, infant morbidity, history of smoking in the home, and maternal parity. Maternal height and weight and selected infant anthropometric measurements were also taken at this time, and again every 3 mo together with data on infant health, infant and young child feeding practices, and morbidity (20).

Stool collection and assessment of parasite status

Stool samples were collected from the infants at 6, 9, and 12 mo of age, and transported in chilled containers to the Parasite Laboratory, Faculty of Medicine, Universitas Padjadjaran, Indonesia. The Kato Katz method (23) was used for microscopic detection of the absence/presence of soil-transmitted helminths and the number of eggs of each of the following species: *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*. No examination was performed for protozoa.

Laboratory assessment

Anticoagulated whole blood and serum from infants were collected from morning, nonfasting, venipuncture blood samples using rigorous trace element–free collection and separation procedures (24) as reported previously (22). Presence of symptoms of infection, time of the blood collection, and time elapsed since the last meal were recorded. Details of the analysis and quality control for complete blood count, serum ferritin, soluble transferrin receptor (sTfR), RBP, zinc, selenium, α 1-acid glycoprotein (AGP), and C-reactive protein (CRP) were reported earlier (22). Briefly, serum ferritin, sTfR, RBP, AGP, and CRP were analyzed by a combined sandwich ELISA technique in the VitMin Laboratory of Dr. J Erhardt, Germany (25), whereas serum zinc and selenium were assayed by inductively coupled plasma MS (Agilent 7500ce ICP-MS; Agilent Technologies) in the Centre for Trace Element Analysis, Department of Chemistry, University of Otago, New Zealand.

Serum vitamin B-12 was analyzed by an automated electrochemiluminescence immunoassay (CV = 4%) using a commercial kit in the

Department of Human Nutrition, University of Otago, New Zealand (vitamin B-12 Elecsys reagent kit, Roche Diagnostics). Accuracy was checked via the manufacturer's controls and values fell within certified ranges. Serum folate was measured by a microbiological assay (CV = 14%) (26, 27), as described previously (28). A high, medium, and low pooled quality control serum control was included on each plate (expressed as mean ± 2SD; "High" 45.5 ± 11.5 nmol/L; "Medium" 26.35 ± 5.2 nmol/L; and "Low" 15.0 ± 3.8 nmol/L) and compared with analyzed values [expressed as mean (%relative standard deviation)] of 49.7 nmol/L (9.7%), 30.3 nmol/L (7.3%), and 15.3 nmol/L (9.7%), respectively. Serum vitamin D (as 25-hydroxyvitamin D) at 6 and 12 mo was analyzed using isotope-dilution LC tandem MS, using an API 3200 instrument (Applied Biosystems) connected to a Dionex Ultimate 3000 HPLC system in the Department of Human Nutrition, University of Otago, New Zealand (29). Analysis of serum 25-hydroxyvitamin D was not performed at 9 mo in view of the low proportion of infants with low concentrations at both 6 and 12 mo and financial constraints. Values for low, medium, and high serum 25-hydroxyvitamin D controls (UTAK Laboratories Inc.) fell within specified ranges, with a CV < 5% for a pooled sample. Baseline maternal finger-prick blood samples were also collected for Hb via the HemoCue Hb 201$^+$ System (HemoCue AB).

For the assay of genetic Hb disorders, RBCs were washed 3 times and divided into aliquots in cryovials for storage at −80°C, before shipment on dry ice to the Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Thailand for analysis by automated cation exchange HPLC (VARIANT II, β-thalassemia short program, Bio-Rad Laboratories) (30).

Values for folate, vitamin D, ferritin, RBP, zinc, and selenium were each adjusted for inflammation using both CRP and AGP following the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia regression approach (22) in view of the reported effects of inflammation on each of these micronutrient biomarkers (31). Anemia was defined as Hb <110 g/L (32), and when accompanied by either low serum ferritin (i.e., <12 μg/L) or high sTfR (i.e., >8.3 mg/L) was considered to reflect IDA. Storage iron depletion was defined as serum ferritin <12 μg/L (33); tissue iron deficiency as sTfR >8.3 mg/L; low RBP as <0.83 μmol/L (25); low serum zinc as <9.9 μmol/L (24); low serum selenium as <0.82 μmol/L (34); low serum vitamin B-12 as <150 pmol/L (35); low serum folate as <6.8 nmol/L (36); and low serum 25-hydroxyvitamin D as <50 nmol/L (37, 38).

Statistical analyses

Data were transferred into Stata version 12 (StataCorp LP) and descriptive and comparative statistics calculated. All continuous variables were plotted and distributions visually assessed. Principal component analysis was used to derive an asset-based wealth index following the Demographic Health Survey Wealth Index guidelines (39). Subsequently, the wealth index was divided into quintiles from the lowest to the highest household wealth. Ferritin, CRP, and AGP were log transformed before analysis. The mean ± SD or geometric mean (95% CI) for Hb, micronutrient biomarkers, and growth indicators was calculated, where appropriate at each age. To allow comparison between all 3 ages, calculations were also performed for those participants with measures at all 3 ages.

TABLE 1 Sociodemographic maternal and infant characteristics[1]

	n	6 mo
Household and maternal factors		
Education	228	
No/primary school		40.8
Secondary school		53.1
Tertiary school		6.1
Age, y	225	27.5 ± 7.2
Smoking at home	217	72.8
BMI,[2] kg/m^2	218	
Underweight		7.3
Normal		56.0
Overweight		30.7
Obese		6.0
Height <145 cm	223	15.3
Hemoglobin, g/L	217	132 (130, 134)
Parity	223	
1		39
2		35.9
≥3		25.1
Infant factors		
Female	228	55.3

[1]Values are percentages, mean ± SD, or geometric mean (95% CI).
[2]Classification of BMI for Asian populations (40): <18.5, underweight; 18.5–24.9, increasing but acceptable risk (normal); 25.0–29.9, increased risk (overweight); and ≥30.0, high risk (obese).

Univariate regression analysis examined the relation between Hb and all predictors using the same respondents at all ages to allow for age-related hypotheses, with the exception of soil-transmitted helminthiasis and genetic Hb disorders. There was only 1 case of *A. lumbricoides* found at 9 mo. The latter were not included because of the small number of infants identified with these disorders and the limitations of the assay method used. Consequently, helminthiasis and genetic Hb disorders were not included in the univariate regression analysis. Sensitivity analyses included all available respondents.

Household, mother, and infant predictors with $P < 0.250$ at any time point were included in a multivariate analysis of the associations between all biomarkers and Hb. Only the iron biomarker (i.e., serum ferritin) and marker of inflammation (i.e., CRP) that explained the highest amount of the variance (R^2) with Hb were included in the multivariate models to avoid issues with multicollinearity. Only the full sample data at 6 and 12 mo were used in the multivariate model to reduce the number of statistical models created, and to enable the vitamin D biomarker, not assessed at 9 mo, to be included. All variables were standardized for multivariate analysis.

Missing micronutrient biomarker values were imputed and sensitivity analyses undertaken. The number of missing values were 22 at 6 mo and 27 at 12 mo for both selenium and zinc, 36 and 32 at 6 and 12 mo for folate, respectively, 48 and 34 at 6 and 12 mo for vitamin B-12, respectively, and 35 and 27 at 6 and 12 mo for vitamin D, respectively. Imputation was performed using multivariate normal regression with an iterative Markov Chain Monte Carlo method for 20 imputations. Model assumptions were checked using residual plots. A significance level of $P < 0.05$ was used for the multivariate models.

TABLE 2 Infant BMI *z* scores, Hb, IDA, micronutrient biomarkers, and proportion with micronutrient deficiencies at 6, 9, and 12 mo of age with no missing values for the respondents at each age[1]

	n	6 mo	9 mo	12 mo
BMI *z* score	188	0.27 ± 1.14	0.23 ± 1.03	0.09 ± 1.08
Hb, g/L	188	112.1 (110.5, 113.7)	110.4 (108.7, 112.1)	110.5 (108.7, 112.4)
Anemia (Hb < 110 g/L)	188	33.5	43.1	38.8
sTfR, mg/L	166	5.8 (5.4, 6.3)	6.9 (6.4, 7.4)	7.5 (7.0, 8.0)
Iron deficiency (sTfR > 8.3 mg/L)	166	16.3	29.5	38.0
IDA based on sTfR	166	14.1	23.5	23.4
Ferritin, μg/L	166	24.1 (21.1, 27.4)	13.0 (11.6, 14.7)	8.6 (7.7, 9.6)
Iron deficiency (ferritin < 12 μg/L)	166	23.5	45.8	66.9
IDA based on ferritin	166	16.0	32.6	36.4
RBP, μmol/L	166	1.00 (0.98, 1.04)	1.07 (1.03, 1.10)	1.07 (1.03, 1.10)
RBP deficiency (<0.83 μmol/L)	166	18.1	8.4	13.2
Zinc, μmol/L	103	12.0 (11.5, 12.5)	11.3 (11.0, 11.6)	11.8 (11.5, 12.2)
Zinc deficiency (<9.9 μmol/L)	103	13.6	17.5	9.7
Selenium, μmol/L	103	0.73 (0.70, 0.75)	0.77 (0.74, 0.79)	0.82 (0.80, 0.85)
Selenium deficiency	103	76.7	60.2	47.6
Vitamin B-12, pmol/L	86	244.3 (217.4, 274.4)	260.1 (233.2, 290.2)	286.3 (236.8, 263.1)
Vitamin B-12 deficiency (<150 pmol/L)	86	17.4	11.6	9.3
Folate, nmol/L	90	48.8 (45.5, 52.4)	49.8 (46.6, 53.2)	50.2 (47.2, 53.4)
Folate deficiency (<6.8 nmol/L)	90	0.0	0.0	0.0
Vitamin D, nmol/L	116	89.7 (85.8, 93.8)	N/A	83.2 (79.5, 87.1)
Vitamin D deficiency (<50 nmol/L)	116	0.9	N/A	4.3

[1]Values are percentages, mean ± SD, or geometric mean (95% CI). Hb, hemoglobin; IDA, iron deficiency anemia; N/A, not available; RBP, retinol-binding protein.

Results

Of the 275 infants who were approached, 230 (83.6%) were recruited and 190 completed the study. Reasons for nonparticipation and withdrawal were described previously (20). Blood samples were provided by all respondents, although the volume of serum obtained was insufficient for some of the assays (22). No significant differences by sex, anthropometric, health, or socioeconomic status were found between those individuals who participated and those who were lost to follow-up.

Of the mothers, about one-third were primiparous, more than half had attended secondary school, and nearly one-third were overweight, with 6% of women classified as obese (**Table 1**). More than two-thirds of the households were exposed to smoking in the home. Mean ± SD BMI *z* score for infants decreased from 0.27 ± 1.14 at age 6 mo to 0.09 ± 1.08 at age 12 mo (**Table 2**). Additional health characteristics of the infants were reported previously (20).

Prevalence of anemia was highest at age 9 mo (43.1%), whereas that of IDA was highest at age 12 mo (23.4% and 36.4% based on sTfR and ferritin, respectively). Serum micronutrient biomarker data at each age are presented in Table 2 for participants with measures at all 3 ages; complete data are presented in **Supplemental Table 1**. There were no significant differences between concentrations of serum vitamin D and folate before and after adjustment for inflammation. Serum vitamin D increased after adjustment for inflammation from 85.8 to 93.8 nmol/L at 6 mo and from 79.5 to 87.1 nmol/L at 12 mo. Serum folate increased after adjustment at 6, 9, and 12 mo from 45.5 to 52.4 nmol/L, 46.6 to 53.2 nmol/L, and 47.2 to 53.4 nmol/L, respectively.

Geometric mean concentrations for all the micronutrient biomarkers varied across the 3 age groups, although the differences were nonsignificant, with the exception of ferritin which decreased significantly from age 6 to 12 mo ($P < 0.001$). Very few infants or none had low concentrations of serum vitamin D (<50 nmol/L) or folate (<6.8 nmol/L), regardless of age, whereas the prevalence of low serum vitamin B-12, RBP, and zinc was between 13.6% and 18.1% at 6 mo, 8.4% and 17.5% at 9 mo, and 9.3% and 13.2% at 12 mo, respectively. Of the micronutrients, serum selenium had the highest prevalence of low values across all ages, followed by ferritin, indicative of depleted iron stores (22).

Only 17.2% of the children at age 6 mo and 10.0% at age 12 mo had no evidence of micronutrient deficiencies. Instead, nearly 50% of the infants (42–43%) had 1 micronutrient deficiency, most frequently iron, and about one-third (28–31%) had 2 micronutrient deficiencies at some time point during the study. The number of nonanemic infants with β-thalassemia, Hb E with α-thalassemia, Hb H, and α-thalassemia was 4, 2, 1, and 14, respectively.

Predictors of Hb and anemia

In the univariate model, maternal height was associated with both Hb and anemia at ages 6 and 9 mo, but only with anemia at 12 mo, whereas infant CRP was significant for Hb and anemia at 6 mo (**Tables 3** and **4**). Ferritin and being female were positively associated with Hb and negatively associated with anemia and sTfR was negatively associated with Hb and positively associated with anemia across all ages. RBP was positively associated with Hb and inversely related to anemia at 9 and 12 mo.

In the multiple regression model, ferritin, tertiary education, higher maternal Hb, lower CRP concentrations, and being female were significantly and positively associated with Hb at 6 mo, whereas only ferritin and RBP were significant and positive predictors of Hb at 12 mo (**Table 5**). The sensitivity analyses that were undertaken with imputed missing data showed no meaningful differences in associations

TABLE 3 Univariate associations with Hb at ages 6, 9, and 12 mo with no missing values for the respondents at each age[1]

		6 mo		9 mo		12 mo	
	n	Mean difference in Hb (g/L) per unit increase (95% CI)	*P* value	Mean difference in Hb (g/L) per unit increase (95% CI)	*P* value	Mean difference in Hb (g/L) per unit increase (95% CI)	*P* value
Household and maternal factors							
Wealth index (quintiles)	183	−0.11 (−1.31, 1.10)	0.860	0.95 (−0.21, 2.10)	0.108[2]	0.55 (−0.74, 1.84)	0.403
Education	188						
No/primary school		Reference		Reference		Reference	
Secondary school		1.93 (−1.34, 5.20)	0.246[2]	0.46 (−3.05, 3.97)	0.796	−0.05 (−3.79, 3.70)	0.980
Tertiary school		5.04 (−1.34, 11.43)	0.121[2]	6.57 (1.29, 11.85)	0.015[2]	4.47 (−3.20, 12.15)	0.252
Maternal age, y	185	−0.08 (−0.28, 0.12)	0.431	0.06 (−0.14, 0.27)	0.542	0.11 (−0.13, 0.34)	0.362
Smoking at home	179	−0.86 (−4.28, 2.55)	0.619	1.58 (−1.98, 5.15)	0.382	0.06 (−3.94, 4.05)	0.977
Maternal BMI, kg/m^2	180	0.05 (−0.39, 0.48)	0.828	0.16 (−0.30, 0.63)	0.486	0.00 (−0.46, 0.45)	0.988
Maternal height, cm	185	−0.44 (−0.72, −0.17)	0.002[2]	−0.36 (−0.68, −0.03)	0.031[2]	−0.20 (−0.54, 0.14)	0.246[2]
Maternal Hb, g/L	178	0.09 (−0.02, 0.20)	0.128[2]	0.09 (−0.02, 0.20)	0.099[2]	0.06 (−0.06, 0.19)	0.337
Parity	183	−0.56 (−1.87, 0.75)	0.399	0.84 (−0.48, 2.16)	0.212[2]	0.87 (−0.50, 2.23)	0.211[2]
ln(CRP), ln(mg/L)	166	−1.00 (−1.77, −0.23)	0.011[2]	−0.77 (−1.75, 0.21)	0.125[2]	−0.46 (−1.53, 0.60)	0.392
ln(AGP), ln(g/L)	166	−2.81 (−5.45, −0.17)	0.037[2]	−1.43 (−4.49, 1.63)	0.357	−1.16 (−4.23, 1.91)	0.456
Infant sex (female vs. male)	188	7.88 (4.96, 10.79)	<0.001[2]	5.83 (2.58, 9.08)	0.001[2]	5.49 (1.95, 9.03)	0.003[2]
BMI *z* score	188	−0.02 (−1.36, 1.32)	0.977	0.87 (−0.76, 2.49)	0.292	−0.33 (−2.10, 1.44)	0.713
Biomarkers							
Folate, nmol/L	90	0.10 (−0.02, 0.23)	0.100	−0.02 (−0.18, 0.14)	0.797	0.04 (−0.14, 0.21)	0.668
Vitamin B-12, pmol/L	84	0.00 (−0.01, 0.01)	0.530	0.01 (−0.00, 0.02)	0.243	0.00 (−0.02, 0.02)	0.825
Vitamin D, nmol/L	116	0.05 (−0.03, 0.13)	0.263	N/A	N/A	0.06 (−0.02, 0.13)	0.168
ln(Ferritin), ln(μg/L)	166	4.74 (2.83, 6.65)	<0.001	7.10 (4.97, 9.22)	<0.001	8.34 (5.80, 10.89)	<0.001
sTfR, mg/L	166	−1.38 (−1.96, −0.81)	<0.001	−1.25 (−1.71, −0.78)	<0.001	−1.27 (−1.74, −0.81)	<0.001
RBP, μmol/L	166	6.23 (−0.36, 12.81)	0.064	14.36 (5.91, 22.80)	0.001	15.47 (7.83, 23.12)	<0.001
Zinc, μmol/L	103	0.10 (−0.41, 0.61)	0.696	−0.44 (−2.10, 1.23)	0.606	−0.31 (−1.35, 0.72)	0.551
Selenium, μmol/L	103	−1.66 (−18.61, 15.29)	0.846	4.16 (−14.16, 22.49)	0.653	−7.82 (−22.49, 6.84)	0.292

[1] AGP, α 1-acid glycoprotein; CRP, C-reactive protein; Hb, hemoglobin; ln, log-transformed; N/A, not available; RBP, retinol-binding protein; sTfR, serum soluble transferrin receptor.
[2] $P < 0.250$ for household and maternal factors.

compared with the original models. The final regression models without imputations for infants at ages 6 and 12 mo explained 37.1% and 35.3% of the variance in Hb, respectively.

The potential predictors for anemia at 6 mo were similar to those reported for low Hb at 6 mo (i.e., ferritin, having a higher CRP concentration, and being male), with the exception of maternal education and the addition of a greater maternal height (**Table 6**). At 12 mo, low concentration of serum ferritin was the only significant independent predictor of anemia.

Discussion

At least one-third of the Sumedang infants, irrespective of age, had anemia, which at 9 and 12 mo nearly reached or surpassed the level indicative of a severe public health problem (i.e., >40%) (31). Serum ferritin (41), after correction for inflammation, was the only biomarker that predicted Hb and risk of anemia at both 6 and 12 mo of age, highlighting the urgent need to improve the iron status of these infants during late infancy. In contrast, the contribution of nonnutritional factors as predictors of low Hb concentrations and anemia was restricted to infants at 6 mo of age. The roles of helminthic infection and genetic Hb disorders were not explored in view of the small number of cases for these 2 conditions. Reports on the prevalence of helminthic infection and genetic Hb disorders in Indonesia, especially among infants, are limited.

Earlier studies in Indonesia (2, 42) also observed that low Hb concentrations during late infancy were associated with ID (based on low serum ferritin), although none to our knowledge examined the potential role of other micronutrients known to have a role in hematopoiesis, unlike here. In this study, a similar relation between low Hb concentrations and low serum ferritin was observed, after adjusting for inflammation, at both 6 and 12 mo of age. This association was attributed, at least in part, to inadequate intakes of dietary iron in the infants at both 6 and 12 mo of age (20). Such deficits occurred even though almost all infants at age 6 mo (91.7%) consumed iron-fortified foods (20). However, both the amount of the iron-fortified products consumed as well as the readily available heme iron from flesh foods were low at 12 mo (20).

Of the nonnutritional factors investigated here, inflammation based on CRP was found to predict a low Hb and be a significant independent risk factor for anemia at age 6 mo. This trend is not unexpected (43), arising in part from disturbances in iron homeostasis in response to high concentrations of circulating hepcidin stimulated by inflammatory

TABLE 4 Univariate associations with anemia at ages 6, 9, and 12 mo with no missing values for the respondents at each age[1]

		6 mo		9 mo		12 mo	
	n	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Household and maternal factors							
Wealth index (quintiles)	183	0.92 (0.74, 1.15)	0.479	0.90 (0.73, 1.11)	0.332	1.01 (0.82, 1.25)	0.930
Maternal education	188						
No/primary school		Reference		Reference		Reference	
Secondary school		0.70 (0.37, 1.31)	0.262	1.13 (0.62, 2.06)	0.688	1.15 (0.62, 2.11)	0.663
Tertiary school		0.28 (0.06, 1.35)	0.112[2]	0.24 (0.05, 1.14)	0.073	0.49 (0.12, 1.91)	0.302
Age, y	185	1.00 (0.96, 1.04)	0.937	1.00 (0.96, 1.04)	0.896	0.99 (0.95, 1.03)	0.663
Smoking at home	179	1.47 (0.71, 3.04)	0.304	0.87 (0.45, 1.69)	0.685	0.99 (0.50, 1.94)	0.969
Maternal BMI, kg/m^2	180	0.99 (0.92, 1.07)	0.842	0.97 (0.90, 1.05)	0.473	1.02 (0.95, 1.10)	0.611
Maternal height, cm	185	1.05 (1.04, 1.17)	0.002[2]	1.07 (1.01, 1.13)	0.023[2]	1.06 (1.00, 1.13)	0.040[2]
Maternal hemoglobin, g/L	178	0.99 (0.99, 1.01)	0.443	0.99 (0.97, 1.01)	0.348	0.98 (0.95, 1.00)	0.029[2]
Parity	183	0.94 (0.71, 1.26)	0.695	0.80 (0.60, 1.07)	0.131[2]	0.83 (0.63, 1.11)	0.215[2]
ln(CRP), ln(mg/L)	166	1.22 (1.01, 1.47)	0.038[2]	1.12 (0.94, 1.33)	0.208[2]	1.04 (0.88, 1.23)	0.635
ln(AGP), ln(g/L)	166	1.42 (0.81, 2.50)	0.222[2]	1.45 (0.85, 2.48)	0.169[2]	1.24 (0.75, 2.06)	0.398
Infant sex (female vs. male)	188	0.24 (0.13, 0.46)	<0.001[2]	0.50 (0.28, 0.90)	0.021[2]	0.45 (0.25, 0.82)	0.009[2]
BMI *z* score	188	0.98 (0.75, 1.28)	0.874	0.80 (0.60, 1.06)	0.124[2]	1.04 (0.79, 1.37)	0.769
Biomarkers							
Folate, nmol/L	90	0.98 (0.96, 1.01)	0.251	1.00 (0.98, 1.04)	0.549	0.96 (0.93, 1.00)	0.028
Vitamin B-12, pmol/L	84	1.00 (1.00, 1.00)	0.861	1.00 (1.00, 1.00)	0.383	1.00 (1.00, 1.00)	0.429
Vitamin D, nmol/L	117	0.99 (0.98, 1.01)	0.498	N/A	N/A	0.99 (0.97, 1.01)	0.216
ln(Ferritin), ln(μg/L)	166	0.47 (0.31, 0.71)	<0.001	0.41 (0.26, 0.65)	<0.001	0.27 (0.16, 0.47)	<0.001
sTfR, mg/L	166	1.26 (1.11, 1.44)	<0.001	1.14 (1.04, 1.24)	0.004	1.16 (1.06, 1.27)	0.001
RBP, μmol/L	166	0.36 (0.08, 1.68)	0.192	0.13 (0.03, 0.56)	0.007	0.21 (0.05, 0.90)	0.036
Zinc, μmol/L	103	0.99 (0.86, 1.13)	0.854	1.18 (0.90, 1.55)	0.225	0.99 (0.80, 1.23)	0.950
Selenium, μmol/L	103	4.30 (0.12, 158.78)	0.429	0.11 (0.00, 2.53)	0.165	2.13 (0.10, 47.20)	0.633

[1]AGP, α 1-acid glycoprotein; CRP, C-reactive protein; ln, log-transformed; N/A, not available; RBP, retinol-binding protein; sTfR, serum soluble transferrin receptor.
[2]$P < 0.250$ for household and maternal factors.

cytokines (44). However, in addition to disturbances in iron homeostasis, increased hepcidin expression induced by inflammation may also contribute to impaired erythropoiesis and slightly shortened RBC survival time which, together with lower Hb, further increase the risk of anemia (45).

At age 6 mo we also observed a negative relation between male sex and Hb, and a higher risk of anemia, a finding consistent with earlier reports in Indonesia (41) and elsewhere (46, 47) and related to sex-related differences in growth rate (41, 48, 49). Certainly, our male and taller infants had greater body weights and thus potentially higher daily requirements for iron than female/smaller infants, which may also be linked with the increased risk of anemia observed at age 6 mo among infants of taller mothers. Not surprisingly, a significant positive correlation existed between maternal height and length-for-age *z* scores of infants ($r = 0.18$; $P = 0.007$), as described elsewhere (50, 51).

Attenuation of the associations between the nonnutritional factors (inflammation and sex) and Hb for the infants by age 12 mo may be linked to the marked depletion in their iron stores at this time compared with the concentrations at age 6 mo when complementary foods first began to replace breast milk. At age 12 mo, 65% of the infants had depleted iron stores (based on ferritin <12 μg/L) compared with only 21% at 6 mo. In New Zealand infants, iron stores (measured by serum ferritin) were a stronger predictor of anemia at age 12 mo than for infants at age 6 mo (46). Seemingly, when iron stores are low, iron becomes the single strongest predictor of Hb after adjusting for other potential nutritional and nonnutritional factors.

Despite seeming deficiencies of vitamin A, B-12, zinc, and selenium across all ages, only vitamin A (assessed by RBP) was positively associated with Hb at age 12 mo, by which time more than two-thirds of the infants had received vitamin A supplements. This finding is not unexpected and has been described elsewhere (11, 52–54).

Our study has several strengths, including a longitudinal design and the collection of many variables associated with low Hb concentrations and anemia during late infancy. We examined 7 micronutrients, adjusted for inflammation where appropriate, and measured nonnutritional factors including inflammation, helminths, and selected genetic Hb disorders. However, identification of the latter among infants at age 12 mo is complicated by the possibility that HbA_2 can rise further for the first 1–2 y of life (55) and IDA can reduce the HbA_2 concentration slightly (55, 56). Other biomarkers related to Hb and iron absorption, such as hepcidin and glucose-6-phosphate dehydrogenase, were also not investigated.

In conclusion, our results emphasize that anemia remains a serious public health problem during infancy in Sumedang district, Indonesia, with low iron status being a major predictor of Hb even after adjustment for several micronutrients and nonnutritional factors. Hence, there is an urgent need to re-evaluate anemia and ID control programs in the district.

TABLE 5 Multivariate associations with infant Hb at 6 and 12 mo of age[1]

Variable, unit	Mean difference in Hb (g/L) per 1-SD increase (95% CI)	*P* value
6 mo ($n = 201$)		
Serum folate, nmol/L	1.53 (−0.52, 3.57)	0.141
Vitamin D, nmol/L	0.61 (−0.87, 2.07)	0.412
ln(Ferritin), ln(μg/L)	3.15 (1.48, 4.83)	<0.001
RBP, μmol/L	1.18 (−0.20, 2.57)	0.093
Zinc, μmol/L	0.23 (−1.75, 2.22)	0.818
Selenium, μmol/L	−0.67 (−2.61, 1.26)	0.493
Vitamin B-12, pmol/L	0.50 (−1.30, 2.30)	0.582
Wealth index (quintiles)	−0.76 (−1.86, 0.33)	0.171
Maternal education		
No/primary school	Reference	
Secondary school	2.33 (−0.90, 5.57)	0.156
Tertiary school	6.64 (0.62, 12.66)	0.031
Maternal height, cm	−0.21 (−0.46, 0.04)	0.103
Maternal Hb, g/L	0.09 (0.00, 0.18)	0.046
ln(CRP), ln(mg/L)	−1.30 (−1.97, −0.63)	<0.001
Sex (female vs. male)	4.98 (1.96, 7.99)	0.001
Parity	−0.30 (−1.76, 1.15)	0.682
12 mo ($n = 169$)		
Serum folate, nmol/L	2.06 (−0.81, 4.93)	0.156
Vitamin D, nmol/L	0.47 (−1.79, 2.74)	0.678
ln(Ferritin), ln(μg/L)	4.38 (2.67, 6.09)	<0.001
RBP, μmol/L	1.66 (0.12, 3.19)	0.035
Zinc, μmol/L	0.82 (−1.49, 3.14)	0.359
Selenium, μmol/L	−0.01 (−2.53, 2.51)	0.995
Vitamin B-12, pmol/L	1.13 (−1.60, 3.87)	0.410
Wealth index (quintiles)	0.35 (−0.89, 1.59)	0.580
Maternal education		
No/primary school	Reference	
Secondary school	−1.12 (−4.97, 2.73)	0.567
Tertiary school	0.86 (−7.96, 9.68)	0.848
Maternal height, cm	−0.11 (−0.46, 0.24)	0.522
Maternal Hb, g/L	−0.00 (−0.12, 0.13)	0.997
ln(CRP), ln(mg/L)	−0.52 (−1.60, 0.52)	0.322
Sex (female vs. male)	2.44 (−1.09, 5.99)	0.175
Parity	−0.12 (−1.71, 1.48)	0.883

[1]CRP, C-reactive protein; Hb, hemoglobin; ln, log-transformed; RBP, retinol-binding protein.

TABLE 6 Multivariate associations with infant anemia at 6 and 12 mo of age[1]

Variable, unit	OR (95% CI)	*P* value
6 mo ($n = 201$)		
Serum folate, nmol/L	0.87 (0.50, 1.49)	0.603
Vitamin D, nmol/L	0.90 (0.61, 1.34)	0.613
ln(Ferritin), ln(μg/L)	0.62 (0.42, 0.90)	0.012
RBP, μmol/L	0.85 (0.58, 1.25)	0.407
Zinc, μmol/L	1.21 (0.70, 2.09)	0.499
Selenium, μmol/L	1.02 (0.68, 1.55)	0.913
Vitamin B-12, pmol/L	1.00 (0.62, 1.61)	0.992
Wealth index (quintiles)	1.08 (0.82, 1.40)	0.596
Maternal education		
No/primary school	Reference	
Secondary school	0.68 (0.31, 1.49)	0.333
Tertiary school	0.19 (0.03, 1.24)	0.083
Maternal height, cm	1.07 (1.00, 1.15)	0.036
Maternal hemoglobin, g/L	0.99 (0.97, 1.02)	0.661
ln(CRP), ln(mg/L)	1.25 (1.02, 1.53)	0.036
Sex (female vs. male)	0.30 (0.14, 0.61)	0.001
Parity	0.84 (0.58, 1.19)	0.323
BMI *z* score	0.85 (0.63, 1.15)	0.290
12 mo ($n = 169$)		
Serum folate, nmol/L	0.57 (0.31, 1.03)	0.064
Vitamin D, nmol/L	0.78 (0.49, 1.23)	0.284
ln(Ferritin), ln(μg/L)	0.45 (0.30, 0.69)	<0.001
RBP, μmol/L	0.83 (0.58, 1.20)	0.328
Zinc, μmol/L	0.90 (0.51, 1.56)	0.697
Selenium, μmol/L	0.90 (0.52, 1.55)	0.700
Vitamin B-12, pmol/L	0.99 (0.59, 1.66)	0.960
Wealth index (quintiles)	1.10 (0.81, 1.49)	0.548
Maternal education		
No/primary school	Reference	
Secondary school	0.89 (0.37, 2.16)	0.796
Tertiary school	0.44 (0.07, 2.94)	0.399
Maternal height, cm	1.05 (0.98, 1.13)	0.195
Maternal hemoglobin, g/L	0.98 (0.95, 1.01)	0.192
ln(CRP), ln(mg/L)	1.04 (0.84, 1.28)	0.739
Sex (female vs. male)	0.73 (0.33, 1.61)	0.441
Parity	0.79 (0.53, 1.18)	0.253
BMI *z* score	0.83 (0.60, 1.17)	0.286

[1]CRP, C-reactive protein; ln, log-transformed; RBP, retinol-binding protein.

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