Determination of Anemia Among Young Children in Rural India

WHAT’S KNOWN ON THIS SUBJECT: The immense burden of anemia among toddlers in developing countries, particularly India, has been documented and attributed to iron deficiency. Limited data are available, however, regarding the biological, nutritional, and socioeconomic etiologies of anemia, especially in rural settings in which the prevalence is maximal.

WHAT THIS STUDY ADDS: We present a comprehensive evaluation in rural Indian children of biological (micronutrient, infectious disease, and genetic), maternal, and socioeconomic factors possibly associated with hemoglobin. In addition to iron status, folate level, maternal hemoglobin level, family wealth/food insecurity, and hemoglobinopathy were also independently associated with hemoglobin.

ABSTRACT

OBJECTIVE: More than 75% of Indian toddlers are anemic. Data on factors associated with anemia in India are limited. The objective of this study was to determine biological, nutritional, and socioeconomic risk factors for anemia in this vulnerable age group.

METHODS: We conducted a cross-sectional study of children aged 12 to 23 months in 2 rural districts of Karnataka, India. Children were excluded if they were unwell or had received a blood transfusion. Hemoglobin, ferritin, folate, vitamin B12, retinol-binding protein, and C-reactive protein (CRP) levels were determined. Children were also tested for hemoglobinopathy, malaria infection, and hookworm infection. Anthropometric measurements, nutritional intake, family wealth, and food security were recorded. In addition, maternal hemoglobin level was measured.

RESULTS: Anemia (hemoglobin level < 11.0 g/dL) was detected in 75.3% of the 401 children sampled. Anemia was associated with iron deficiency (low ferritin level), maternal anemia, and food insecurity. Children’s ferritin levels were directly associated with their iron intake and CRP levels and with maternal hemoglobin level and inversely associated with continued breastfeeding and the child’s energy intake. A multivariate model for the child’s hemoglobin level revealed associations with log(ferritin level) (coefficient: 1.20; P < .001), folate level (0.05; P < .01), maternal hemoglobin level (0.16; P < .001), family wealth index (0.02; P < .05), child’s age (0.05 per month; P < .005), hemoglobinopathy (−1.51; P < .001), CRP level (−0.18; P < .001), and male gender (−0.38; P < .05). Wealth index and food insecurity could be interchanged in this model.

CONCLUSIONS: Hemoglobin level was primarily associated with iron status in these Indian toddlers; however, maternal hemoglobin level, family wealth, and food insecurity were also important factors. Strategies for minimizing childhood anemia must include optimized iron intake but should simultaneously address maternal anemia, poverty, and food insecurity. Pediatrics 2010;126:e140–e149

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KEY WORDS

anemia, India, child preschool, iron-deficiency anemia, public health, poverty, food security

ABBREVIATIONS

WHO—World Health Organization
NFHS—National Family Health Survey
PHC—primary health center
INR—Indian rupees
RBP—retinol-binding protein
CRP—C-reactive protein
CI—confidence interval

www.pediatrics.org/cgi/doi/10.1542/peds.2009-3108
doi:10.1542/peds.2009-3108
Accepted for publication Mar 26, 2010
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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).
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FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.
The World Health Organization (WHO) has estimated that, globally, 1.62 billion people are anemic, with the highest prevalence of anemia (47.4%) among preschool-aged children; of these 293 million children, 89 million live in India. The third National Family Health Survey (NFHS) 2005–2006 revealed that at least 80% of Indian children aged 12 to 23 months were anemic. Anemia was especially prevalent among children in selected villages (PHCs), the basic units of health care delivery in rural India. The Gumballi PHC in the Chamarajnagar district, 112 miles south of Bangalore, serves 21 700 people in 13 villages. The Sugganahalli PHC in the Ramnagara district, 56 miles northwest of Bangalore, serves 14 400 people in ~80 villages. Both districts have agrarian economies and per-capita annual incomes (Chamarajnagar, Indian rupees [INR] 22 006 [US $478]; Ramnagara, INR 26 009 [US $565]) that reflect agrarian economies and per-capita annual incomes (Chamarajnagar, Indian rupees [INR] 22 006 [US $478]; Ramnagara, INR 26 009 [US $565]) that reflect the state and national averages (Karnataka overall, INR 26 123 [US $567]; India overall, INR 25 825 [US $567]).

Iron deficiency is believed to be the most important cause of anemia among children in India and is attributable to poor nutritional iron intake and low iron bioavailability. Other factors, including folate and vitamin B12, iron deficiency anemia is a public-health priority, because anemia is associated with impaired cognitive and psychomotor development. Iron deficiency is believed to be the most important cause of anemia among children in India and is attributable to poor nutritional iron intake and low iron bioavailability. Other factors, including folate and vitamin B12, and A deficiencies, malaria infection, hookworm infestation, and hemoglobinopathies, are also associated with childhood anemia. To our knowledge, no previous report in the published literature has described the relative contribution of these factors to anemia in rural Indian children. To effectively control this problem, health care providers must have a comprehensive understanding of the etiologic factors associated with anemia.

We hypothesized that low hemoglobin concentrations in rural Indian children primarily result from micronutrient (especially iron) deficiencies attributable to poor nutritional intake compounded by adverse socioeconomic conditions and food insecurity. To test this hypothesis, we conducted laboratory, nutritional, anthropometric, and socioeconomic evaluations in a cross-section of rural Indian children aged 12 to 23 months.

PATIENTS AND METHODS

Study Site and Participants

Study participants were members of eligible sample populations in villages served by 2 primary health centers (PHCs), the basic units of health care delivery in rural India. The Gumballi PHC in the Chamarajnagar district, 112 miles south of Bangalore, serves 21 700 people in 13 villages. The Sugganahalli PHC in the Ramnagara district, 56 miles northwest of Bangalore, serves 14 400 people in ~80 villages. Both districts have agrarian economies and per-capita annual incomes (Chamarajnagar, Indian rupees [INR] 22 006 [US $478]; Ramnagara, INR 26 009 [US $565]) that reflect the state and national averages (Karnataka overall, INR 26 123 [US $567]; India overall, INR 25 825 [US $567]).

We randomly selected 3 of 4 subcenters of each PHC. Lists of children living in the villages were compiled from lists obtained from both PHCs and from Anganwadi (child care centers for preschool-aged children), and the information was confirmed by investigators who conducted house-to-house visits. All children aged 12 to 23 months in selected villages were eligible, unless the child was acutely unwell or had received a blood transfusion. The detailed study methods have been published.

Study Procedures

The food-security questionnaire module was adapted from the Household Food Insecurity Access Scale, which has been validated for use in settings in developing countries. The scale covers perceptions about food insecurity and enables calculation of a score from 0 (no food insecurity) to 27 (maximum food insecurity). The Wealth Index, an estimation of household wealth in which assets are assigned a weighted score (maximum of 63), was adapted from the third NFHS. Study participants used containers of standardized sizes for a 24-hour dietary recall to estimate nutrient intake, which was expressed as a percentage of the Indian recommended daily intake.

Information on continued and previous breastfeeding practices was obtained with specific questions about whether the child was currently breastfeeding, the duration of exclusive breastfeeding, and the child’s age at introduction of complementary foods and age at breastfeeding cessation. Each child’s length (from the crown of the head to the heel) (Seca 210 [Seca, Hamburg, Germany] and weight (Seca 872) were measured. Height for age, weight for age, and weight for length were calculated. For these variables, results with z scores less than —2 were defined as stunting, underweight, and wasting, respectively, in accordance with the 2008 WHO child growth standards.

Mothers underwent field estimation of capillary hemoglobin level (HemoCue 201+ [HemoCue, Angelholm, Sweden]). Venous blood (3 mL) was drawn from each child, processed appropriately in the PHC laboratory within 6 hours of collection, and then packed with ice and transported to the reference laboratory. Samples were analyzed within 48 hours of collection.

Laboratory Assays

Laboratory assays were performed as follows: automated complete blood examination (Sysmex XT-2000i [Sysmex Inc, Kobe, Japan]) (used only for hemoglobin estimation); serum ferritin, folate, and vitamin B12 (electrochemiluminescent immunoassay, ELECSYS 2010 [ELECSYS, Hitachi High Technologies Corporation, Tokyo, Japan]; reagents from Roche Diagnostics [Penzberg, Germany]), retinol-binding protein (RBP), high-sensitivity C-reactive protein (CRP) (nephelometry,
Siemens BN-Prospec Nephelometer (Siemens, Marburg, Germany), and hemoglobin variant (high-performance liquid chromatography, Biorad D10 [Biorad Laboratories Inc, Hercules, CA]). Thick and thin blood films were prepared by use of the Jaswant-Singh-Bhattacherji method and evaluated for malaria parasites by technicians for the National Malaria Control Program. For study participants for whom stool samples were returned (n = 142), stool was evaluated microscopically for hookworm ova by use of wet mounts.24

Definitions

Anemia was defined as a hemoglobin level of <11 g/dL in children, <12 g/dL in nonpregnant women, and <11 g/dL in pregnant women, on the basis of WHO definitions.9 Iron deficiency was defined as a ferritin level of <12 ng/mL, or <30 ng/mL if the CRP level was >5 mg/L.9 Using the manufacturer’s reference ranges, we defined vitamin B₁₂ deficiency as serum vitamin B₁₂ level of <210 pg/mL and folate deficiency as a serum folate level of <3.3 ng/mL. Biochemical evidence of inflammation was defined as a CRP level of >5 mg/L25 and β-thalassemia trait as a hemoglobin A₂ level of >3.5%.26 Although RBP level is highly correlated with serum retinol levels, reference ranges in the pediatric population are unclear and cutoffs were not applied.27,28 Malaria was diagnosed if plasmodium parasites (trophozoites, schizonts, or gametocytes) were identified in serum. Results for hookworm ova in stool samples were expressed as present or absent.

Ethics Considerations

Information obtained through community consultation was used to formulate the study design and procedures. Plain-language statements explaining the study were provided to and written informed consent was obtained from the guardians of all child participants. The study was approved by the ethics committees of St John’s National Academy of Health Sciences, Bangalore, India, and the Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Australia.

Statistical Methods

Data were entered into Epi Info 3.4.3 (US Centers for Disease Control and Prevention, Atlanta, GA) and exported to Stata 9 (Stata Corp, College Station, TX) for analysis. A sample size of 390 ensured a ±5% range for the 95% confidence intervals (CIs) of estimates of prevalence. The study had 80% power to detect regression coefficients between continuous variables for which the coefficient was at least 0.1 and the correlation between variables was at least 0.2. Linear regression performed by using continuous variables retained maximum information29; in particular, hemoglobin level was analyzed (rather than “anemia”) as the outcome variable, because the threshold for defining anemia has age and ethnic ambiguities.30 CRP level was analyzed as an ordered categorical variable. Associations between risk factors and outcomes were first evaluated by using univariate linear regression. A multiple-regression model was then iteratively developed. Variables were retained if the P value for their coefficient remained <.05. Standardized (β) coefficients (coefficient standardized with a mean of 0 and SD of 1) were calculated. An R² value was used to determine the variation in hemoglobin level revealed by the model. The Shapiro-Wilk test was used to determine if residual values for the model had a normal distribution.

RESULTS

Between August and October 2008, 88.3% of 470 eligible children living in the selected villages were recruited (Fig 1).17 Mean values with 95% CIs in
pajure are shown unless other- wise indicated.

**Demographics and Food Insecurity**
The mean age of children was 17.2 months, and 204 of 405 (50.3%) were boys. Mean maternal age was 23.2 years, and 45 of 376 mothers (12%) were pregnant. Overall socioeconomic status was low, with a mean wealth index of 18 (17.0–19.0). The median food insecurity score was 3 (0–27), and more than half of the mothers (212 of 402 [52.7%]) reported some degree of household food insecurity during the previous month. Food insecurity and wealth indices were inversely associated (Spearman rank correlation: −0.65; P < .001).

**Nutritional Intake**
The mean nutritional intake for children is shown in Table 1. Mean iron intake from non–breast milk sources consumed during the previous 24 hours was 1.4 (1.3–1.5) mg, which was 11.2% of the recommended daily intake for Indian children. Children who were still breastfed had lower iron intake for complementary food (1.10 mg). Mean (95% CI)

### TABLE 1 Mean Nutrient Intake From Non–Breast Milk Sources During the Previous 24 Hours

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean (95% CI)</th>
<th>RDI</th>
<th>Mean Intake</th>
<th>% RDI</th>
<th>% Consuming &lt;75% RDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron, mg/24 h</td>
<td>1.4 (1.3–1.5)</td>
<td>12</td>
<td>11.7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Folate, mg/24 h</td>
<td>33.8 (31.4–36.3)</td>
<td>30</td>
<td>112.7</td>
<td>27.2 (22.7–31.7)</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂, mg/24 h</td>
<td>0.31 (0.30–0.36)</td>
<td>0.2</td>
<td>147.6</td>
<td>48.6 (41.6–51.6)</td>
<td></td>
</tr>
<tr>
<td>Vitamin A, mg/24 h</td>
<td>131.0 (113.1–151.8)</td>
<td>350</td>
<td>37.4</td>
<td>65.5 (60.8–70.3)</td>
<td></td>
</tr>
<tr>
<td>Energy, kcal/24 h</td>
<td>415.0 (353.9–436.1)</td>
<td>1240</td>
<td>33.5</td>
<td>98.5 (97.2–99.7)</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval; RDI, recommended daily intake.

### TABLE 2 Mean Indices of Hemoglobin Level and Associated Factors

<table>
<thead>
<tr>
<th>Hemoglobin (n = 401), g/dL</th>
<th>9.75 (9.59 to 9.91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (n = 396), ng/mL</td>
<td>10.97 (10.09 to 11.92)</td>
</tr>
<tr>
<td>Vitamin B₁₂ (n = 396), pg/mL</td>
<td>420.1 (403.0 to 437.9)</td>
</tr>
<tr>
<td>Folate (n = 396), ng/mL</td>
<td>9.88 (9.54 to 10.22)</td>
</tr>
<tr>
<td>RBP (n = 382), g/L</td>
<td>0.028 (0.026 to 0.030)</td>
</tr>
<tr>
<td>CRP (n = 396), mg/mL</td>
<td>0.91 (0.77 to 1.06)</td>
</tr>
</tbody>
</table>

Mean (95% CI)

**Anthropometry**

Mean growth indices are shown in Table 2. Almost one-third of the children were underweight (129 of 400 [32.3%] (range: 27.7%–36.9%)). Stunting was seen in 115 of 401 children (28.7% [24.2%–33.1%]), and wasting was seen in 83 of 400 (20.8% [16.8%–24.7%]) (Table 3). Length-for-age z scores between boys and girls were similar, but absolute length was greater in boys (77.8 vs 75.6 cm; P < .001).

### Anemia and Associated Conditions

Hemoglobin levels and biological factors possibly associated with hemoglobin levels are shown in Table 2. Anemia was detected in 75.3% of the children (hemoglobin level: 9.75 g/dL [9.59–9.91]). Anemia was prevalent to a similar extent in nonpregnant women (63.3%; hemoglobin level: 11.2 g/dL [11.0–11.4]) and pregnant mothers (61.0%; hemoglobin level: 10.6 g/dL [10.1–11.1]). Childhood iron deficiency (ferritin level: <12 or <30 mg/mL if CRP level was >5 mg/mL) was seen in 61.9% (57.1%–66.7%). Children with anemia were more likely to have iron deficiency (odds ratio [OR]: 6.1; P < .001), maternal anemia (OR: 1.9; P < .01); continued breastfeeding (OR: 1.6; P < .05), and food insecurity (OR: 2.2; P < .005) compared with children without anemia (Table 3).

### Associations With Hemoglobin

Hemoglobin was positively associated with child’s age (coefficient: 0.05 [0.00–0.10]; P < .05) and was lower in boys compared with girls (t test, mean difference in hemoglobin: −0.51 g/dL [−0.19 to −0.82]; P < .01). Univariate regression analysis, controlled for age and gender, revealed that hemoglobin level was positively associated with ferritin, folate, and vitamin A intake; wealth; and maternal hemoglobin level and negatively associated with food insecurity (Table 4). Multiple regression analysis results indicated that children’s hemoglobin levels were primarily associated with ferritin intake but also positively associated with maternal hemoglobin level, child folate intake and age, and family wealth and were inversely associated with male gender, CRP level, and presence of the β-thalassemia trait (Table 5). The food insecurity score could be substituted for wealth index without affecting other factors. The residuals for this model were normally distributed (Shapiro-Wilk test, P > .05), and the R² value was 0.51.
TABLE 3 Proportion of Children With Anemia (Hemoglobin <11 g/dL) and Associated Conditions

<table>
<thead>
<tr>
<th>Factor</th>
<th>Overall, n/N</th>
<th>% (95% CI)</th>
<th>Anemic, % (95% CI)</th>
<th>Not Anemic, % (95% CI)</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory indices</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>245/396</td>
<td>61.9 (57.1 to 66.7)</td>
<td>72.2 (67.1 to 77.3)</td>
<td>29.9 (20.6 to 39.2)</td>
<td>6.1 (3.6 to 10.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>5/396</td>
<td>1.3 (0.2 to 2.4)</td>
<td>1.0 (−0.1 to 2.1)</td>
<td>2.1 (−0.8 to 4.3)</td>
<td>0.5 (0.1 to 5.9)</td>
<td>.42</td>
</tr>
<tr>
<td>Vitamin B₁₂ deficiency</td>
<td>11/396</td>
<td>2.8 (1.2 to 4.4)</td>
<td>2.7 (0.1 to 4.5)</td>
<td>3.1 (−0.0 to 6.5)</td>
<td>0.9 (0.2 to 5.1)</td>
<td>.628</td>
</tr>
<tr>
<td>Inflammation</td>
<td>39/396</td>
<td>9.9 (6.9 to 12.9)</td>
<td>10.7 (7.2 to 14.2)</td>
<td>7.2 (2.1 to 12.4)</td>
<td>1.5 (0.6 to 4.3)</td>
<td>.317</td>
</tr>
<tr>
<td>Hemoglobinopathy</td>
<td>5/401</td>
<td>1.3 (0.2 to 2.3)</td>
<td>1.7 (0.2 to 3.1)</td>
<td>0.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hookworm infestation</td>
<td>19/142</td>
<td>13.4 (7.7 to 19.0)</td>
<td>16.2 (8.9 to 23.4)</td>
<td>7.5 (−0.7 to 15.7)</td>
<td>2.4 (0.6 to 13.4)</td>
<td>.178</td>
</tr>
<tr>
<td>Malaria infection</td>
<td>0/0</td>
<td>—</td>
<td>0.0</td>
<td>0.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nutritional intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low iron intake</td>
<td>386/386</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Continued breastfeeding</td>
<td>234/404</td>
<td>57.9 (53.1 to 62.8)</td>
<td>60.9 (55.4 to 66.4)</td>
<td>49.0 (39.1 to 58.9)</td>
<td>1.6 (1.0 to 2.6)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Food insecurity</td>
<td>212/402</td>
<td>52.7 (47.8 to 57.6)</td>
<td>57.1 (51.5 to 62.8)</td>
<td>38.1 (28.3 to 48.0)</td>
<td>2.2 (1.3 to 3.6)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Maternal hemoglobin level: maternal anemia</td>
<td>227/360</td>
<td>63.1 (58.1 to 68.1)</td>
<td>66.8 (61.2 to 72.4)</td>
<td>51.1 (40.8 to 61.6)</td>
<td>1.9 (1.1 to 3.2)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Child growth</td>
<td>Stunting</td>
<td>115/401</td>
<td>28.7 (24.2 to 33.1)</td>
<td>28.2 (24.1 to 34.4)</td>
<td>26.5 (17.6 to 35.4)</td>
<td>1.1 (0.7 to 2.0)</td>
</tr>
<tr>
<td>Underweight</td>
<td>128/400</td>
<td>32.3 (27.7 to 36.9)</td>
<td>32.6 (27.2 to 37.9)</td>
<td>30.9 (21.6 to 40.3)</td>
<td>1.1 (0.6 to 1.8)</td>
<td>.76</td>
</tr>
<tr>
<td>Wasting</td>
<td>83/400</td>
<td>20.8 (16.8 to 24.7)</td>
<td>19.9 (15.3 to 24.4)</td>
<td>22.9 (14.4 to 31.5)</td>
<td>0.8 (0.5 to 1.5)</td>
<td>.52</td>
</tr>
</tbody>
</table>

Indicates confidence interval.
* Serum folate level < 3.3 ng/mL.
* Serum vitamin B₁₂ level < 210 pg/mL.
* CRP level > 5 mg/L.
* Hemoglobin A₂ > 3.5%.
* Parasites identified on microscopy.
* Iron intake >75% of Indian recommended daily intake.
* The child was continuing to receive breast milk at the time of the study.
* Household Food Insecurity Access Scale score ≥1.
* Hemoglobin level <12 g/dL, or hemoglobin level <11 g/dL if pregnant.
* Length-for-age z score less than −2.
* Weight-for-age z score less than −2.
* Weight-for-length z score less than −2.

Associations With Ferritin

By using univariate regression we found that child’s (log)ferritin level was associated with maternal hemoglobin level (coefficient: 0.09 [0.03–0.13]; P < .01) and CRP level (coefficient: 0.18 [0.12–0.25]; P < .001) but not with child’s age, family wealth, food insecurity, or child’s nutrient intake. According to multiple regression analysis results, child’s log(ferritin) was independently, positively associated with maternal hemoglobin level (coefficient: 0.07 [0.02–0.12]; P < .01), CRP level (coefficient: 0.20 [0.13–0.27]; P < .01), and log(iron intake) (coefficient: 0.25 [0.01–0.49]; P < .05). On the other hand, child’s log(ferritin) was negatively associated with a history of breastfeeding beyond 12 months (coefficient: −0.24 [−0.42 to −0.06]; P < .01) and increased calorie intake from complementary foods (coefficient: −0.001 [−0.002 to −0.000]; P < .02).

DISCUSSION

We made the following observations among 12- to 23-month-old rural Indian children. (1) Hemoglobin levels in children were primarily related to iron stores. (2) Levels of hemoglobin were also associated with levels of folate, CRP, and the β-thalassemia trait. (3) Hemoglobin levels were independently associated with maternal hemoglobin level, family wealth, and food insecurity. (4) Ferritin level was positively associated with dietary-iron intake and inversely associated with continued breastfeeding beyond 1 year of age and increased energy intake from complementary foods. Taken together, these data identify major determinants of childhood anemia in rural Indian toddlers and raise questions regarding anemia-control policies.

The association between child’s hemoglobin level and child’s iron status and maternal hemoglobin level may have multiple pathways (Fig 2). For instance, antenatal anemia contributes to low birth weight and prematurity, both of which increase the risk of childhood anemia.31 Severe maternal anemia may also reduce breast milk iron content.32 Children’s iron intake in this population is universally low, particularly in children continuing to breastfeed. Calories are predominantly available in cereals, which contain inhibitors of iron absorption.34 Thus, increased caloric intake may be associated with reduced dietary-iron bioavailability.34 Finally, the mother and child share a socioeconomic envi-
Although growth may contribute to the development of anemia in this age group, we, similar to other investiga-
tors, did not identify an association between growth and anemia. Measurement of children’s growth trajectories may have helped us identify such an association, but unfortunately, birth records were generally unavailable or unreliable. Our data did con- firm previously reported associations between lower hemoglobin levels and male gender, findings that were possi-
ably related to greater absolute longitudinal growth among boys.

The risk of iron-deficiency anemia may thus depend on complex interactions between dietary-iron content (type of diet), iron bioavailability (duration of breastfeeding and appropriate comple-
mentary feeding practices), increased iron use (growth velocity and erythropoietic mass expansion), and inap-
propriate iron losses (infection and infes-
tation).

Other biological factors associated with childhood hemoglobin levels included serum folate level, presence of inflammation, and hemoglobinopathy status. We identified associations between hemoglobin and CRP levels only when we included ferritin levels in the regression equation; higher CRP levels decreased the coefficient of the relation-
ship between hemoglobin and ferritin levels, a result related to ferritin being an acute-phase protein. Al-
though vitamin A intake was often low and was associated with hemoglobin levels, serum RBP level was not associ-
ated with hemoglobin level, a finding that may be related to a successful government program to supplement vitamin A.

The results of our study highlight impor-
tant associations of wealth and food in-
security with anemia that, although pre-
viously reported, are independent of other measured environmental and bio-
logical factors. This observation sug-
gests that broader socioeconomic con-
ditions directly influence hemoglobin levels in children. Potential explanations

### TABLE 4 Regression Coefficients (Univariate) Between Hemoglobin Level and Conditions in the Child

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient (95% CI)**</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximate factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin**</td>
<td>1.17 (1.02 to 1.32)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Folate</td>
<td>0.08 (0.03 to 0.12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vitamin B₁₂**</td>
<td>0.07 (−0.31 to 0.45)</td>
<td>.706</td>
</tr>
<tr>
<td>RBP**</td>
<td>0.17 (−0.12 to 0.45)</td>
<td>.250</td>
</tr>
<tr>
<td>β-Thalassemia traitd</td>
<td>−0.76 (−2.20 to 0.88)</td>
<td>.301</td>
</tr>
<tr>
<td>CRP**</td>
<td>0.01 (−0.12 to 0.13)</td>
<td>.897</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length for age, z score</td>
<td>0.05 (−0.09 to 0.19)</td>
<td>.493</td>
</tr>
<tr>
<td>Weight for age, z score</td>
<td>0.04 (−0.11 to 0.20)</td>
<td>.588</td>
</tr>
<tr>
<td>Weight for length, z score</td>
<td>0.02 (−0.13 to 0.17)</td>
<td>.826</td>
</tr>
<tr>
<td>Distal factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wealth</td>
<td>0.02 (0.01 to 0.04)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Food insecurity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score 1–5</td>
<td>−0.08 (−0.70 to 0.53)</td>
<td>.789</td>
</tr>
<tr>
<td>Score 6–10</td>
<td>−0.66 (−1.02 to −0.30)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Score 11–15</td>
<td>−0.94 (−1.68 to −0.19)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Score ≥16</td>
<td>−0.24 (−0.80 to 0.33)</td>
<td>.411</td>
</tr>
<tr>
<td>Maternal education</td>
<td>0.10 (−0.01 to 0.22)</td>
<td>.083</td>
</tr>
<tr>
<td>Maternal literacy</td>
<td>0.18 (−0.17 to 0.54)</td>
<td>.311</td>
</tr>
<tr>
<td>Maternal hemoglobin level</td>
<td>0.28 (0.18 to 0.37)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Child’s birth order</td>
<td>−0.11 (−0.32 to 0.09)</td>
<td>.280</td>
</tr>
<tr>
<td>Iron intake</td>
<td>0.16 (0.07 to 0.43)</td>
<td>.158</td>
</tr>
<tr>
<td>Folate intake</td>
<td>0.00 (−0.00 to 0.01)</td>
<td>.193</td>
</tr>
<tr>
<td>Vitamin B₁₂ intake</td>
<td>0.37 (−0.14 to 0.87)</td>
<td>.157</td>
</tr>
<tr>
<td>Vitamin A intake</td>
<td>0.17 (0.05 to 0.27)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Child still breastfeedingf</td>
<td>−0.28 (−0.62 to 0.06)</td>
<td>.108</td>
</tr>
</tbody>
</table>

The regression coefficients were controlled for age and gender.

* Logarithmically transformed.

** The child’s age (in months) and gender were included in each regression equation but not reported separately; age and gender were associated with hemoglobin in all equations.

A positive result for β-thalassaemia minor was coded as 1; all other results were coded as 0.

The results for CRP level were categorized as 0, 0.1–1, 1–3, 3–5, 5–10, and >10 mg/L.

The n values for the food insecurity scores were 190 (score 0), 30 (score 1–5), 123 (score 6–10), 21 (score 11–15), and 49 (score ≥16).

The results were coded as 1 if the child was still breastfeeding or 0 if the child was fully weaned.

### TABLE 5 Multiple Regression Model of Associations With Hemoglobin

<table>
<thead>
<tr>
<th>Association With Hemoglobin</th>
<th>Coefficient (95% CI)**</th>
<th>Standardized Coefficienta</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(ferritin), ng/mL**</td>
<td>1.20 (1.06 to 1.35)</td>
<td>0.64</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum folate, ng/mL</td>
<td>0.05 (0.01 to 0.09)</td>
<td>0.11</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>CRP, mg/L**</td>
<td>−0.18 (−1.06 to 1.35)</td>
<td>−0.14</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>β-Thalassemia traitd</td>
<td>−1.51 (−2.53 to −0.48)</td>
<td>−0.11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Maternal hemoglobin, g/dL</td>
<td>0.16 (0.08 to 0.23)</td>
<td>0.17</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wealth</td>
<td>0.02 (0.00 to 0.03)</td>
<td>0.10</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Genderc</td>
<td>−0.38 (−0.62 to −0.13)</td>
<td>−0.12</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Age of child, mo</td>
<td>0.05 (0.02 to 0.09)</td>
<td>0.11</td>
<td>&lt;.005</td>
</tr>
</tbody>
</table>

a The regression coefficient was standardized with a mean of 0 and an SD of 1.

* Logarithmically transformed.

The results for CRP level were categorized as 0, 0.1–1, 1–3, 3–5, 5–10, and >10 mg/L.

A positive result for β-thalassaemia minor was coded as 1; all other results were coded as 0.

Gender was coded as male (1) or female (0).
include generalized bone marrow failure because of malnutrition, deficiencies in other micronutrients, exposure to biofuel smoke, and possibly other unexplained mechanisms associated with lower socioeconomic status. We were surprised that we found no association between the highest level of food insecurity and hemoglobin level (Table 4), a result that may have been related to the nonlinear performance of the Household Food Insecurity Access Scale.

Major consequences of climate change are impairments of crop yield and agricultural productivity, conditions that could increase food insecurity and worsen childhood anemia. Furthermore, the continuing global financial crisis may threaten the health status of low- and middle-income countries and may play a role in childhood anemia through its effect on food insecurity. Thus, childhood anemia may worsen if the stressors listed above undermine socioeconomic advancement or worsen food insecurity in India. Incorporation of strategies to support nutrition and address socioeconomic conditions may help mitigate these phenomena.

Few studies conducted worldwide have comprehensively examined the etiology of anemia in children in the infant-to-toddler age group. Although to our knowledge there have been no studies of anemia etiology in rural India, a study in urban slums of New Delhi investigated 90 anemic children and identified important contributions from iron and vitamin B_{12} deficiency. A study of young Mexican children revealed that anemia attributable to iron-deficiency anemia was less common than anemia from other causes. In Malawi, infectious diseases and vitamin B_{12} and folate deficiencies, but not iron deficiency, were important factors associated with severe childhood anemia. Results of studies in Thailand and the United States also indicated that iron deficiency was a nondominant cause of pediatric anemia. In addition to confirming the findings of the NFHS (which was a large prevalence study), our data provide major insights into biological, sociodemographic, and economic factors associated with anemia in rural toddlers. The contrast between our findings of predominant iron-deficiency anemia and the findings reported in the published literature may reflect differences in diet and socioeconomic patterns in this area and also in study methods.

The findings of our study should be considered with awareness of the following limitations. First, this is a cross-sectional study, for which we report association rather than causation. Second, measurement of additional laboratory variables, particularly levels of soluble transferrin receptor, methylmalonic acid, and homocysteine, could have increased the detection of iron deficiency and functional folate and vitamin B_{12} deficiencies. Furthermore, we did not evaluate levels of lead or selenium, which have been previously demonstrated to be associated with anemia. These assays were prohibited because the amount of blood they require exceeded the maximum phlebotomy volume acceptable to the community. Third, incomplete stool sampling in the field may have resulted in failure to detect an association between hookworm infestation and hemoglobin or ferritin levels. Finally, the 24-hour dietary-recall method we used has limitations, and may have led to overestimation of nutritional intake in young children compared with methods that use weight measurement. However, this method was the tool that could be most feasibly administered within the
available time frame to assess nutritional intake in our field setting. Despite these limitations, we have identified a comprehensive set of factors associated with hemoglobin levels in rural Indian toddlers. The high level of community participation, identification of anemia prevalence that reflects nationwide prevalence, and socioeconomic similarities of the selected districts of Karnataka with other states in India suggest that the results of this study may be generalizable to much of India and perhaps to other resource-limited settings in Asia. Iron-deficiency anemia, a leading risk factor for burden of disease in developing countries, is associated with impaired cognitive development and potentially restricts economic development. Globally, policy makers have deployed strategies against anemia that include iron supplementation, food fortification, and dietary diversification. Although the Indian anemia-control program recommends that children younger than 5 years receive iron and folic acid supplements, our study results show that this approach has not successfully controlled anemia prevalence. This apparent lack of success may be related to suboptimal program implementation, lack of adherence, or other unidentified causes. Thus, additional work is required to identify reasons for the gap between policy and practice for anemia control in this setting. The findings of our study support the need for a broad public-health strategy for the control of anemia among Indian children beyond delivering iron supplementation alone. Measures that address maternal anemia could have functional and reproductive benefits for mothers and, subsequently, children. The recent WHO recommendation to provide weekly iron and folic acid to all women of reproductive age could be expanded in rural India. Low dietary-iron intake, particularly in breastfeeding children, ideally should be alleviated with a combined approach of iron supplementation, fortification of complementary foods, and dietary education. These efforts must be coupled with strategies to address family poverty and food security, because both are independently associated with hemoglobin levels in children.

CONCLUSIONS

Anemia, an important problem worldwide, is increasing among young children in India and requires urgent attention. Our findings suggest that current public-health strategies such as iron supplementation are necessary but not sufficient to reduce childhood anemia. Instead, combining iron supplementation and food-fortification programs with efforts to reduce maternal anemia, family poverty, and food insecurity may yield optimal improvement of children’s hemoglobin levels.

ACKNOWLEDGMENTS

This study was supported by grant funding from the Allen Foundation, Michigan (to Dr Shet) and funds from the Fred P. Archer Charitable Trust, Victoria, Australia, and the Melbourne Research Scholarship, University of Melbourne, Victoria, Australia (to Dr Pasricha). We acknowledge the field team, led by Mrs Varalaxmi Vijaykumar, which was involved in data collection. We thank Ms Shubha K. for assistance with data entry. We are indebted to the community health workers and village Anganwadi workers who assisted with our field work. We are grateful to Dr Julie Simpson for guidance with the statistical analysis. We thank Professor Rob Moodie for guidance in developing the study.

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Determinants of Anemia Among Young Children in Rural India
Pediatrics; originally published online June 14, 2010;
DOI: 10.1542/peds.2009-3108

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Determinants of Anemia Among Young Children in Rural India

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