



Applied nutritional investigation

Prevalence and predictors of anemia in a population of North Indian children

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ABSTRACT

Objective: Anemia is an important health concern worldwide, particularly in poor populations such as in India. The objective of this study was to determine the prevalence and predictors of anemia and iron status.

Methods: One thousand children ages 6 to 30 mo were included in a study undertaken in low- to middle-income neighborhoods in New Delhi, India. Children of Tigri and Dakshinpuri were identified through a community survey. Plasma concentrations of hemoglobin (Hb), soluble transferrin receptor (sTfR), folate, vitamin B₁₂, and total homocysteine (tHcy) were measured. Predictors for plasma Hb concentration were identified in multiple linear regression models and considered significant if *P*-value < 0.05.

Results: The prevalence of anemia (Hb concentration < 11 g/dL) was 69.6% (*n* = 696) whereas the prevalence of iron deficiency (elevated sTfR i.e., > 4.7 nmol/L) was 31% (*n* = 309). The main predictors for Hb concentration were plasma concentrations of sTfR (standardized beta coefficient [β], −0.49; *P* < 0.001), folate (β , 0.15; *P* < 0.001), vitamin B₁₂ (β , 0.10; *P* < 0.001), tHcy (β , −0.11; *P* < 0.001) among the biomarkers. Length-for-age Z score (β , 0.08; *P* = 0.002) and family income (β , 0.06; *P* = 0.027) also predicted Hb concentration.

Conclusion: Anemia was common in this population. Iron, folate, and vitamin B₁₂ status were important predictors for plasma Hb concentration. Improving the status of these nutrients might reduce the burden of childhood anemia in India.

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Introduction

Anemia is an important health concern, particularly in poor populations such as India. More than 1.6 billion people are anemic worldwide including almost half of all preschool children, and one-third of these 300 million preschool children live

in India [1]. The third National Family Health Survey estimated that approximately 79% of Indian preschool children were anemic [2]. Rural areas have generally been more affected than urban locations [3,4]. Despite an impressive economic development in India, particularly in the urban areas, the proportion of the population that is anemic seems to remain stable and has even increased in recent years [5,6]. It has been established that approximately half of all anemia cases are due to iron deficiency [2,7,8]. Iron deficiency is the most prevalent nutritional disorder in the world today and is also believed to be the most important cause of anemia among children in India [9]. It is related to poor nutritional iron intake, low iron bioavailability, and low socioeconomic status [10,11]. Other nutrients such as folate and vitamin B₁₂ also probably play a role in childhood anemia, and deficiency of these nutrients also may result in anemia [12–14]. In this study of 1000 children from a low socioeconomic area of

ST, TAS, and NB designed the research. ST, TK, FA, MM, and SM conducted the research. CSY was involved in analysis of plasma specimens. BK was responsible for data management and data analysis. TK, ST, BK, and TAS analyzed the data and prepared the manuscript. TAS had primary responsibility for final content. All authors read and approved the final manuscript and there was no conflict of interest between the authors, and with the funding agencies.

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New Delhi, India we measured hemoglobin (Hb) concentrations to assess the prevalence of anemia. We also measured other biomarkers such as iron, vitamin B₁₂, and folate and used these and clinical, socioeconomic, and anthropometric variables to identify predictors for the Hb concentration.

Materials and methods

Study site and participants

The study was conducted in the low to middle socioeconomic settings of Tigr and Dakshinpuri in New Delhi with a total population of about 300 000. Details of the population have been described previously [15,16]. The present findings come from the baseline data of a randomized double-blind, placebo-controlled preventive field trial with a factorial design that evaluated the effect of supplementation with folic acid, vitamin B₁₂, or both on childhood infections. The results of the study have been published elsewhere [17].

Procedures

By means of a door-to-door survey, 1377 children ages 6 to 30 mo of either sex were identified and 1000 were enrolled in the study between January 2010 and September 2011 [17]. Children with severe systemic illness requiring hospitalization, severe malnutrition (weight for height z score [WHZ] <−3 Z), or severe anemia (Hb < 7 g/dL), those taking folic acid and/or vitamin B₁₂ supplements, and those not consenting, or moving away were excluded from enrollment (Fig. 1).

Weight was measured using digital Digitron scales with 50 g sensitivity (Digitron, S n S, Delhi, India). Length was measured using locally manufactured infantometer reading to the nearest 0.1 cm (Nikhil traders, Delhi, India).

Laboratory parameters

A blood sample (~3 mL) was obtained for all children and collected in EDTA-containing vacutainers (BD, Franklin Lakes, NJ, USA). The blood was centrifuged at ~450 g at room temperature for 10 min, plasma was separated and transferred into storage vials, and stored at −20°C until analysis (Remi Sales & Engineering Ltd, Mumbai, India). Blood samples were analyzed for Hb concentration using HemoCue AB (HemoCue Hb Angelholm, Sweden). The HemoCue analyzer has been used extensively worldwide for estimating the concentration of Hb with capillary blood in field conditions, and has been found to provide accurate results, comparable to estimates from more sophisticated laboratory instruments [18]. The instrument has been validated against major automatic cell counters and was found to agree well with all tested systems [19]. Plasma concentration of folate and vitamin B₁₂ were estimated by microbiologic assays using a chloramphenicol resistant strain of *Lactobacillus casei* [20] and colistin sulphate resistant strain of *Lactobacillus leichmannii*, respectively [21]. Plasma soluble transferrin receptor (sTfR; marker of iron status) was analyzed quantitatively using an immunoturbidimetric assay from Roche diagnostics on the Roche modular P800 (Roche diagnostics, Basel, Switzerland) [22] and plasma total

homocysteine (tHcy) was analyzed using commercial kits (Abbott Laboratories, Abbott Park, IL, USA) [23].

Definitions

Anemia was defined as Hb <11 g/dL on the basis of World Health Organization (WHO) criteria [1,8]. Iron deficiency was defined as sTfR concentrations >4.7 nmol/L [24]. We defined vitamin B₁₂ deficiency as plasma vitamin B₁₂ <200 pmol/L and folate deficiency as a plasma folate <7.5 nmol/L [17,25].

Questionnaires used for the data collection were developed and compiled by experienced study investigators. All the questionnaires were pretested in the field, standard operating procedures were made, and dummy questionnaires were filled before administered to the study staff and participants.

A team of educated, skilled, and experienced fieldworkers, lab technicians, supervisors, and physicians collected the data. The study team went through extensive training sessions before the study start and only those who had reached a certain level of skills were allowed to collect data. Supervised and non-supervised visits were made at regular intervals.

The study staff was standardized in their respective tasks at the beginning as well as at regular intervals during the trial in activities such as blood collection techniques, anthropometrics, and temperature and respiratory rate measurements. Standardization sessions were repeated every 6 mo. The instruments used in the study were calibrated at regular intervals throughout the study period.

Ethics

This study was conducted according to the latest version of the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committees at Society for Essential Health Action and training New Delhi, Society for Applied Studies New Delhi, Christian Medical College Vellore, and Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST) before the initiation of the study. 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 involved in the study. Information consent forms were translated in a simple local language (Hindi) and those who were unable to read, the staff obtaining consent read out the information sheet in the presence of an impartial literate witness and those who were unable to sign, a thumb imprint was taken, witnessed (countersigned) by an impartial literate witness. If the caregiver wanted to keep a copy of the form to show to other literate family members before consenting, a copy was left with the caregiver and the caregiver was asked to come on next day for consenting.

Statistical analysis

The forms used for data collection were designed in visual basic.net in computer [26]. Double data entry by two data entry clerks followed by validation was completed within 72 h. Range and consistency checks were incorporated. Continuous variables were reported as means or medians and categorical variables as proportions in the baseline table. The statistical analyses were performed with Stata, version 12 (StataCorp, College Station, TX, USA).

Linear regression was used to identify predictors for Hb concentration. The associations were first evaluated in crude linear regression models and then in multiple-regression models in a stepwise process. Variables were retained in the multiple regression models if the *P*-value for their coefficient remained <0.05. Three different statistical models were developed. The first model contained the demographic, clinical, and anthropometric variables; the second model contained only the biomarkers; and in third model included all variables of the previous two models. Because of covariability between tHcy and vitamin B₁₂, these two variables were not included in the statistical models simultaneously. Standardized beta coefficients (β) were calculated for the estimation of the strength of the associations. Biomarkers of folate, vitamin B₁₂, and iron status, as well as other variables, were log-transformed to achieve normality when necessary. We used generalized additive model (GAM) plots in the package “mgcr” in the statistical software R [27,28] to depict the “dose-response” relationship between sTfR, tHcy, vitamin B₁₂, and folate concentration with Hb concentration. In the GAM models, biochemical variables were adjusted for age, breast-feeding status, family income, possession of items, and length-for-age (HAZ) Z score.

Results

One thousand of the 1377 screened children were found eligible for the study and enrolled. The baseline characteristics of the enrolled children are shown in Table 1. The mean age of

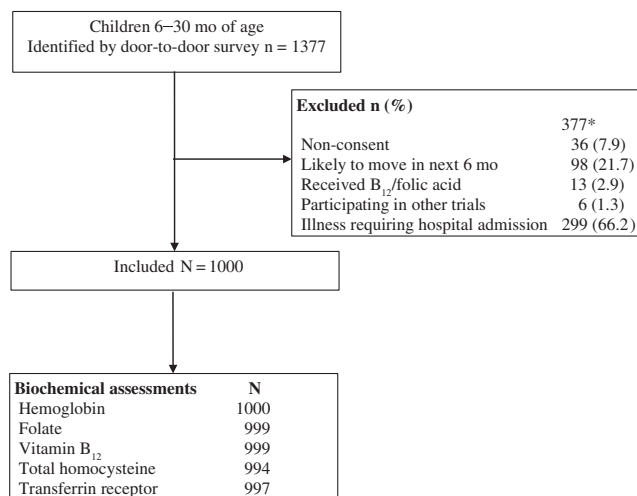


Fig. 1. Study profile.

Table 1

Baseline characteristics of children ages 6 to 30 mo included in the analysis

| Variables | n | Mean | SD |
|--|------|-------|----------------|
| Child characteristics | 1000 | | |
| Age (mo) | | 16.1 | 7 |
| 6–11 mo (%) | 321 | 32.1 | |
| 12–23 mo (%) | 484 | 48.4 | |
| 24–30 mo (%) | 195 | 19.5 | |
| Boys (%) | 507 | 50.7 | |
| Breastfed (%) | 723* | 72.3 | |
| Family situation | | | |
| Annual income (median/IQR) (INR) | | 75500 | 60 000–144 000 |
| Families who own color TV, scooter, or cooler (%) | 882 | 88.2 | |
| Maternal characteristics[†] | | | |
| Age | | 25.8 | 4 |
| Years of schooling | | 6.7 | 5 |
| Mothers who work (%) | 86 | 8.6 | |
| Paternal characteristics | | | |
| Years of schooling | | 8.8 | 4 |
| Fathers who work (%) [†] | 980 | 98.1 | |
| Household characteristics | | | |
| Nuclear family (%) | 537 | 53.7 | |
| Family size (median/IQR) | | 5 | 4–7 |
| Nutritional status | | | |
| Weight (kg) | | 8.5 | 2 |
| Length (cm) | | 74.2 | 7 |
| Z score weight for length (wasted), ≤ 2 (%) | 105 | 10.5 | |
| Z score length for age (stunted), ≤ 2 (%) | 425 | 42.5 | |
| Z score weight for age (underweight), ≤ 2 (%) | 341 | 34.1 | |
| Morbidity | | | |
| Prevalence in previous 24 h (%) | | | |
| Diarrhea [†] | 52 | 5.2 | |
| Cough or difficult breathing or fast breathing | 320 | 32.0 | |

INR, Indian National Rupees; IQR, interquartile range

* n is 995, missing data of 5 children.

† n is 999, missing data of 1 child.

enrolled children was (16.1 ± 7.1 mo) and 51% were boys. Around 72% of children were breastfed. Mean maternal age was (25.8 ± 4.2 y), and 86 (8.6%) mothers were working outside home. Median annual income of the family was (75 500, interquartile range 60 000–144 000) rupees. Mean weight at baseline was (8.5 ± 1.6 kg) and mean length was (74.2 ± 7.0 cm). More than one-third of the children were underweight. Stunting was seen in 42.5% of the children and 10.5% were wasted. Approximately 5% of the children had experienced diarrhea in the previous 24 h and one-third had either cough, or difficult or fast breathing.

Mean Hb concentration of the children in this population was 10.1 ± 1.4 g/dL and 70% had an Hb concentration < 11 g/dL. Iron deficiency (sTfR > 4.7 nmol/L) was seen in 31%. The median concentrations of sTfR, tHcy, vitamin B₁₂, and folate are shown in Table 2.

The variables that we selected for the linear regression models are listed in Table 3 and the adjusted regression coefficients (Coef) are shown in Table 4. We categorized the variables into child characteristics and biochemical variables and measured the association between these variables and Hb concentration in three multiple regression models. Model 1 shows that older children had an average lower Hb concentration than infants (Coef, -0.48 ; 95% confidence interval [CI], -0.68 to -0.28 for age group 12–23 mo and Coef, -0.40 ; 95% CI, -0.66 to -0.14 for age group 24–30 mo, reference group of infants [ages 6–11 mo]). Breastfed children had higher mean Hb concentrations compared with those who were not breastfed (Coef, 0.34; 95% CI, 0.14–0.54 for breastfed children). All biomarkers were

Table 2

Hb concentration and biomarkers status in children ages 6 to 30 mo

| Variables | N | Mean | SD |
|---|------|--------|------------|
| Hb status | | | |
| Hb concentration (g/dL) | 1000 | 10.1 | 1.4 |
| Number of children with Hb < 11 g/dL (%) | 696 | 69.6 | |
| Number of children with Hb < 10 g/dL (%) | 458 | 45.8 | |
| Biomarkers status | | | |
| | N | Median | IQR |
| Plasma folate concentration (nmol/L) | 997 | 11 | (6.6–20.4) |
| Number of children with plasma folate concentration < 7.5 nmol/L (%) | 301 | 30.2 | |
| Plasma vitamin B ₁₂ concentration (pmol/L) | 998 | 266 | (176–405) |
| Number of children with plasma vitamin B ₁₂ concentration < 200 pmol/L (%) | 327 | 32.8 | |
| Plasma tHcy concentration (μ mol/L) | 994 | 11.5 | (8.9–15.9) |
| Number of children with plasma tHcy concentration > 10 μ mol/L (%) | 628 | 63.1 | |
| Plasma sTfR nmol/L | 997 | 3.6 | (2.6–5.3) |
| Number of children with plasma sTfR > 4.7 nmol/L (%) | 309 | 31.0 | |

Hb, hemoglobin; IQR, interquartile range; sTfR, soluble transferrin receptor; tHcy, total homocysteine

significantly associated with the Hb concentration (model 2). In model 3, the most saturated model, the variables that were significantly associated with Hb concentration were as follow: Plasma sTfR (β , -0.49 ; $P < 0.001$), plasma folate (β , 0.15; $P < 0.001$), plasma vitamin B₁₂ (β , 0.10; $P < 0.001$) and plasma tHcy (β , -0.11 ; $P < 0.001$) of the biomarker variables and Z score HAZ (β , 0.08; $P = 0.002$) of the growth indicators. Family income also predicted (β , 0.06; $P = 0.027$) Hb concentration.

The associations of most of the demographic and nutritional variables were substantially attenuated from model 1 to model 3, the full model. This is an indication that the associations described in model 1 were confounded by iron or vitamin statuses. The estimates from the biochemical markers, however, did not change substantially from model 2 to 3 when the demographic and nutritional variables were included.

The clinical and socioeconomic variables in model 1 explained 11% of the variability of the Hb concentration and the biochemical variables in model 2 (iron status, vitamin B₁₂, and folate status) explained 34% of the variability. Adding model 1 to model 2 only increased the explained variance from 34% to 36%. Plasma sTfR concentration was the most important predictor for Hb concentration in our study. Figure 2 depicts the associations between Hb concentrations and sTfR (a), folate (b), vitamin B₁₂ (c), and tHcy (d) concentrations. The solid lines show how the relationship between the biomarker covaries with the Hb concentration. The shaded area shows the 95% CI of this association. The Hb concentration was reasonably linearly associated with all four biomarkers and the values of these associations were similar to those generated from the multiple regression models described in Table 4.

Discussion

Our study showed that more than of two-thirds of the children were anemic, which is consistent with other studies from India [2,29]. The main predictor for Hb concentration, and thus for anemia, was plasma sTfR concentration, which is an indicator of iron stores in the body and its level in the blood increases when Hb concentration decreases. This confirms that iron is one of the most important determinants for anemia. Folate status (measured by plasma folate) and vitamin B₁₂ status (measured by plasma concentrations of vitamin B₁₂ and tHcy) also predicted

Table 3

The variables assessed in the multivariate regression models as predictors for the Hb concentration

| Variables | Continuous | Categorical | | |
|--|-----------------|-------------------------|------------------------------|-------------------|
| Breast-feeding status | – | 0 = Non-breastfed (NBF) | 1 = Breastfed (BF) | – |
| Age | Months | 0 = if age <12 mo | 1 = if age ≥12 mo and <24 mo | 2 = if age ≥24 mo |
| Sex | – | 3 = Boy | 4 = Girl | – |
| Years of schooling, mother | Years | – | – | – |
| Years of schooling, father | Years | – | – | – |
| Members in the household | Number | – | – | – |
| Maternal age | Years | – | – | – |
| Maternal working status | – | 0 = Not working | 1 = Working | – |
| Father working status | – | 0 = Not working | 1 = Working | – |
| Weight-for-length Z score (WHZ) | Z scores | – | – | – |
| Length-for-age Z score (HAZ) | Z scores | – | – | – |
| Weight-for-age Z score (WAZ) | Z scores | – | – | – |
| Annual family income (INR) | Log-transformed | – | – | – |
| Family type | – | 3 = Nuclear | 4 = Joint | – |
| Family assets (color TV, cooler, or scooter) | – | 1 = Yes | 2 = No | – |
| Diarrheal illness (prevalence in last 24 h) | – | 1 = Yes | 2 = No | – |
| Hb concentration | Unit | – | – | – |
| Plasma folate concentration | Log-transformed | – | – | – |
| Plasma vitamin B ₁₂ concentration | Log-transformed | – | – | – |
| Plasma tHcy concentration | Log-transformed | – | – | – |
| Plasma sTfR concentration | Log-transformed | – | – | – |

Hb, hemoglobin; INR, Indian National Rupees; sTfR, soluble transferrin receptor; tHcy, total homocysteine

plasma Hb concentration [30,31]. Plasma folate and Vitamin B₁₂ showed a positive association with Hb concentration, whereas tHcy was negatively associated. The log-transformed markers of all these three micronutrients were reasonably linearly associated with the concentration of Hb as shown in Figure 2.

We did not measure serum ferritin and used sTfR as an indicator of iron status. Raised plasma sTfR concentration has been considered as the most useful indicator of a functionally significant iron deficit reflecting poor iron stores [32] and sTfR has a higher diagnostic efficacy than serum ferritin [33]. Furthermore, sTfR is less affected than serum ferritin by inflammation and infections [34,35] and is accordingly even more reliable than ferritin when inflammation is present [36].

In our study, severe infection was an exclusion criteria, but it is possible that we could have overlooked children with

infections who had few or mild symptoms. We did not measure any marker of acute or subacute inflammation because plasma was limited, and because we used plasma sTfR rather than ferritin as a marker for iron status. Furthermore, inflammation is an important cause of anemia in children in poor countries and could have explained the high proportion of anemic children in this population. Unfortunately, there are no good markers of chronic inflammation that could have been used to in this setting. It is possible that inflammation contributes to explaining the reminding two-thirds of the variability of the Hb concentration.

Our analysis showed that older children had on average lower Hb concentrations than younger children. Older children also had poorer iron status and when the iron status was taken into account in the multiple regression models, the “age effect” on

Table 4

Summary of multiple linear regression analysis for variables predicting Hb concentration

| Variables | Model 1 | | | Model 2* | | | Model 3* | | |
|----------------------------------|---------|----------------|---------|----------|----------------|---------|----------|----------------|---------|
| | Coef | 95% CI | P-value | Coef | 95% CI | P-value | Coef | 95% CI | P-value |
| Child characteristics | | | | | | | | | |
| Age | | | | | | | | | |
| 6–11 mo | Ref | | | | | | Ref | | |
| 12–23 mo | –0.48 | –0.68 to –0.28 | <0.001 | | | | –0.07 | –0.25–0.11 | 0.453 |
| 24–30 mo | –0.40 | –0.66 to –0.14 | 0.003 | | | | –0.03 | –0.26–0.20 | 0.815 |
| Breast-feeding | 0.34 | 0.14–0.54 | 0.001 | | | | 0.16 | –0.02–0.35 | 0.082 |
| Family economic status | | | | | | | | | |
| Annual income [†] | 0.24 | 0.11–0.36 | <0.001 | | | | 0.12 | 0.01–0.23 | 0.027 |
| Possession of items [‡] | –0.35 | –0.62 to –0.07 | 0.013 | | | | –0.27 | –0.50 to –0.04 | 0.022 |
| Nutritional status | | | | | | | | | |
| HAZ | 0.17 | 0.10–0.25 | <0.001 | | | | 0.10 | 0.04–0.17 | 0.002 |
| Biomarkers^{§,} | | | | | | | | | |
| Plasma folate | | | | 0.35 | 0.25–0.45 | <0.001 | 0.28 | 0.17–0.38 | <0.001 |
| Plasma vitamin B ₁₂ | | | | 0.26 | 0.13–0.39 | <0.001 | 0.24 | 0.10–0.37 | 0.001 |
| Plasma tHcy [¶] | | | | –0.39 | –0.55 to –0.22 | <0.001 | –0.35 | –0.54 to –0.16 | <0.001 |
| Plasma sTfR | | | | –1.28 | –1.41 to –1.15 | <0.001 | –1.22 | –1.35 to –1.08 | <0.001 |
| R-squared | | 0.11 | | | 0.34 | | | 0.36 | |
| Observations | | 1000 | | | 995 | | | 995 | |

CI, confidence interval; Coef, coefficient; HAZ, length/height for age Z score; Hb, hemoglobin; sTfR, soluble transferrin receptor; tHcy, total homocysteine

* In model 2 and 3, tHcy and vitamin B₁₂ were not included simultaneously in the analysis because of covariability between these two variables.

† Log-transformed variable.

‡ Families owning color TV, scooter, or cooler.

§ All the biomarkers in the model 2 and model 3 were log transformed.

|| Standardized beta coefficients: sTfR (–0.49), folate (0.15), vitamin B₁₂ (0.10), tHcy (–0.11), HAZ (0.08), and family income (0.06) in model 3.¶ Values of tHcy are given when vitamin B₁₂ was not included in the analysis.

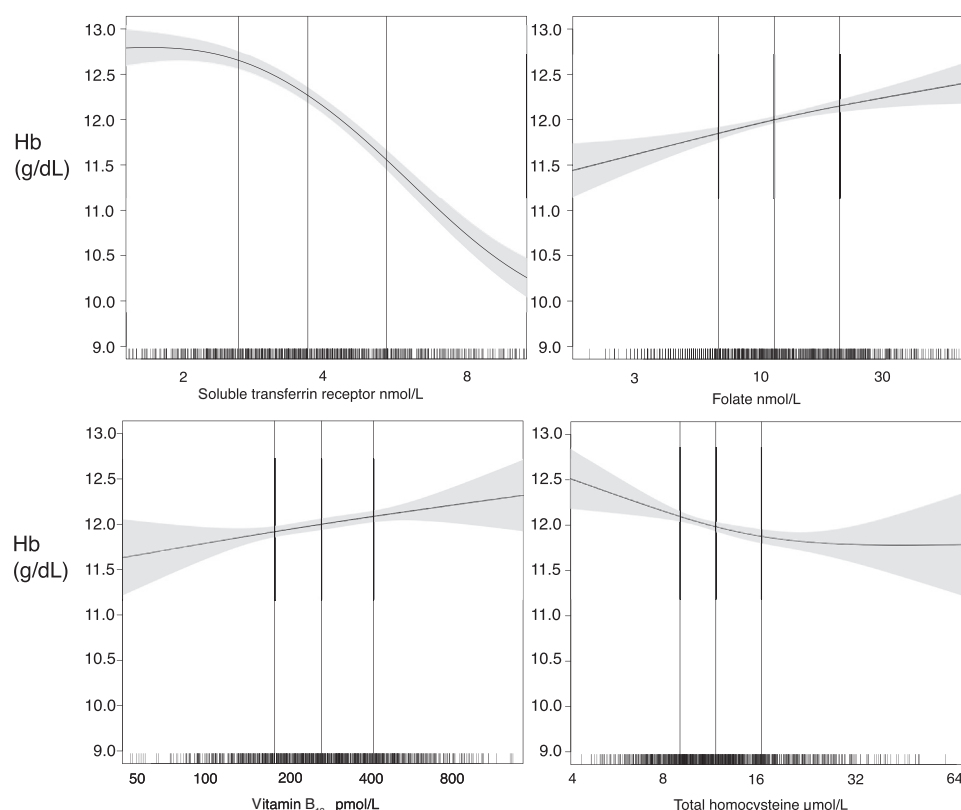


Fig. 2. Associations between Hb concentration and sTfR, folate, vitamin B₁₂, and tHcy. The graphs were constructed using generalized additive models in R, the solid line depicts the association of the outcome and the independent variable. The shaded area spans the 95% confidence interval of this association.

the Hb concentration disappeared. Breastfed children had on average higher Hb concentration than non-breastfeed children. This association was substantially attenuated when adjusting for vitamin and iron status in model 3. Thus, this is an indication that breast-feeding reduces the risk for nutritional anemia even in children older than 6 mo of age. Children from poor families were more anemic and reflecting that anemia is a problem related to poverty.

We also found that many children had poor vitamin B₁₂, poor folate, or poor status of both in the approximately 1000 children in whom we undertook the biochemical analyses (Table 2). Almost one-third had a folate concentration <7.5 nmol/L and about one-third had a vitamin B₁₂ concentration <200 pmol/L (Table 2). Both nutrients are important for cell division and differentiation and poor status of either of these can result in macrocytic anemia [37], such as in pernicious anemia. Macrocytic anemia is also common in patients on antifolate treatment [38]. Vitamin B₁₂ deficiency was an important predictor for severe anemia in Malawian children, except for this study; our study is to our knowledge the first study demonstrating vitamin B₁₂ as an important predictor for Hb concentration in young children [13]. A high proportion with poor vitamin B₁₂ status is not surprising as many of the families in this population (including young children) only eat vegetarian diets and because of the cost, the intake of animal source foods is low even among the non-vegetarian families [25,39,40]. Poor folate status is an indication that the intake of fruits and leafy vegetables is inadequate. In fact, vegetable intake among the population in India is much lower than the WHO/Food and Agricultural Organization recommended level [41]. These children reside in the city of New

Delhi but their access to fresh fruits and vegetables is probably limited. In a recent survey in India, more than half of the lower middle-class families said they are unable to purchase fresh fruits and vegetables because of “skyrocketing prices”, particularly in major cities such as New Delhi [42].

Our findings support the recommendations of providing iron and folic acid to children who are anemic [11,43]. Although the government of India recommends folic acid and iron for children ages 6 mo to 5 y, but this program is not effective. Action should be taken to effectively implement this program to reduce the prevalence of childhood anemia in India [44,45]. Such efforts could also include novel methods to improve iron status as suggested by results from a recent study in Andhra Pradesh, India. That study showed significant improvements in body iron stores and reduction in iron deficiency in primary school-aged children when they were given micronized ferric pyrophosphate (fortified extruded rice kernels mixed with normal rice) through midday meal for 8 mo [46].

Whether administration of vitamin B₁₂ and/or folic acid will improve the Hb concentration on a population level, however, is not known and has to be examined in clinical trials. Plasma tHcy is a sensitive marker for both vitamin B₁₂ and folate status. We have previously shown that vitamin B₁₂, but not folate predicted Hcy concentration in this population [25,47]. This was confirmed in this study, in which the vitamin B₁₂ concentration explained 40% of the variability of the tHcy concentration. This is an indication that the poor vitamin B₁₂ status has metabolic consequences that again can result in impaired cell division [48], which can explain the association between poor vitamin B₁₂ status and Hb concentration.

This is not a random sample of the population as all children who could participate in the study were included until we reached the targeted 1000 children. Inclusion and exclusion criteria were defined for a clinical trial; we screened children to reach the desired sample size. The main reasons for not including children were ongoing illness; the chance that they were likely to move away during the study period; and non-consent (Fig. 1). Although illness was a temporary exclusion criteria, most of those who were not enrolled on the first encounter were still not enrolled after recovery from the excluding illness. Children who are ill may be more likely to suffer from nutritional disorders including vitamin and iron deficiencies. Children of migrating families also may be different from those who reside more permanently in the slum areas. Thus, generalizing beyond this study population should be done with caution. However, we believe that the findings in this study are representative for slum dwellers in large north Indian cities. Nonetheless, other populations with different dietary patterns will probably have different nutritional constraints. Because of economic challenges and increasing food prices, families are getting less nutritive foods as shown in a study on Egyptian children [49]. Anemia may have consequences for cognitive development, school achievements, and later work performance and productivity [50]. Despite a massive effort to reduce the burden of anemia and a booming economy, especially in urban areas of India, a large proportion of children are still anemic. It is important to reduce the burden of anemia in most low- and middle-income countries. To reduce the burden of anemia, the existing health systems, health professionals and workers, as well as caregivers need to be involved.

Conclusion

Improving iron status is probably the most important measure toward reducing the burden of anemia. Improving the intake of other nutrients such as folate and vitamin B₁₂ is also likely to be important. However, the role of these nutrients in anemia needs to be verified in other populations within and outside India.

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