Clinical Nutrition xxx (2016) 1–6

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

Supplementation of vitamin B12 or folic acid on hemoglobin concentration in children 6–36 months of age: A randomized placebo controlled trial

Tivendra Kumar [a], Sunita Taneja [b], H.P.S. Sachdev [c], Helga Refsum [d], Chittaranjan S. Yajnik [e], Nita Bhandari [b], Tor A. Strand [f, g, *], Study Group[1]

[a] Society for Essential Health Action and Training, New Delhi, India
[b] Society for Applied Studies, New Delhi, India
[c] Department of Pediatrics, Sitaram Bhartia Institute of Science and Research, New Delhi, India
[d] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway
[e] Diabetes Unit, King Edward Memorial Hospital, Rasta Peth, Pune, India
[f] Innlandet Hospital Trust, Lillehammer, Norway
[g] Centre for International Health, University of Bergen, Norway

ARTICLE INFO

Article history:
Received 2 February 2016
Accepted 6 July 2016

Keywords:
Anemia
Hemoglobin
Iron
Cobalamin
Folate
Homocysteine

SUMMARY

Background & aims: The main objective of this report is to measure to what extent folate or vitamin B12 given daily for 6 months to young North Indian Children improves hemoglobin (Hb) concentration.
Methods: In a randomized placebo controlled trial in low-to-middle income neighborhoods in New Delhi, India, children were randomized into four groups in a 1:1:1:1 ratio and supplemented daily for 6 months with 2 RDAs of vitamin B12, folic acid, both, or placebo. All children with anemia at baseline were given iron supplementation daily for 2 months. We measured the plasma concentrations of soluble transferrin receptor (sTfR), folate, vitamin B12, total homocysteine (tHcy) and Hb in 262 children.
Results: Mean Hb concentration decreased in all four study groups during the six months of follow up and supplementation of either or both of the vitamins did not improve the Hb concentration. Iron supplements for the initial 2 mo had limited effect on anemia at 6 mo as almost 90% were still anemic at study end.
Conclusion: Supplementation of folic acid and/or vitamin B12 for 6 months does not improve Hb concentration in young children. Our findings do not argue for widespread vitamin B12 or folic acid supplementation to combat anemia. Our results also call for alternative strategies to improve iron status and treat iron deficiency anemia.
Clinical trial registry: NCT00717730 at www.clinicaltrials.gov, CTRI No.: CTRI/2010/091/001090 at www.ctri.nic.in.

1. Introduction

Globally, almost 500 million non-pregnant women and 293 million young children suffer from anemia [1]. In the third Indian National Family Health Survey (NFHS), it was estimated that around 79% of Indian preschool children were anemic [2] and the prevalence of anemia have remained stable or slightly increased in recent years despite impressive economic development in India [3,4]. Rural and poor populations are generally more affected than urban and affluent areas [5,6]. Worldwide, approximately half of all anemia cases are due to iron deficiency [1,7] but deficiencies of other nutrients such as folate and vitamin B12 are also common and probably play a role in childhood anemia [8–10]. In a recent report from this study in India, we found that folate and vitamin B12 status along with iron were associated with anemia [11]. However, the impact of routine supplementation of either or both of these vitamins on Hb concentration in children is not known. We

List of abbreviations: WHO, World Health Organization; SD, standard deviation; IQR, interquartile range; Coef, regression.

* Corresponding author. Innlandet Hospital Trust, Anders Sandvigsgate 17, 2629 Lillehammer, Norway.
E-mail address: tors@me.com (T.A. Strand).

[1] Study Group: Sanjana Brahmawar Mohan, Farhana A Rafiqi and Baljeet Kaur.

http://dx.doi.org/10.1016/j.clnu.2016.07.002
0261-5614/

undertook this randomized, placebo-controlled trial to examine the effect of routine vitamin B12 or folic acid supplementation for six months on infections [12]. This manuscript reports on the effect of the interventions on the Hb concentration and iron status at end of study which were predefined secondary outcomes of this study.

2. Materials and methods

2.1. Study site and participants

The study was conducted in a low-to-middle socioeconomic setting of Tigri and Dakshinpuri in New Delhi with a total population of about 300,000. Details of the population have been described previously [13,14]. The present findings come from a randomized double-blind placebo-controlled preventive field trial with a factorial design that evaluated the impact of supplementation with folic acid, vitamin B12, or both on childhood infections [12]. The analyses are restricted to the group with both baseline and end-of-study blood samples, i.e., a total of 262 children.

2.2. Procedures

Of 1377 children aged 6–30 months of either sex identified through a door-to-door survey, 1000 were enrolled in the study conducted between January 2010 and September 2011. Children with severe systemic illness requiring hospitalization, severe malnutrition (WHZ < -3Z), or severe anemia (Hb < 7 g/dL), those taking folic acid and/or vitamin B12 supplements, and those not consenting or considering migration were excluded from enrollment [12].

WHO guidelines were followed for anemia treatment, and the children with severe anemia (Hb < 7 g/dL) were referred to hospitals for further evaluation and treatment. Children with modest anemia (between Hb 11 g/dL and 7 g/dL) were included in the study, but provided iron supplement solution (Ferium, Emcure Pharmaceuticals Ltd) containing elemental iron of 50 mg per 5 mL. Two bottles of the Ferium were provided if the child was above 1 year and one bottle if the age of the child was below 1 year. The dose was doubled when the child crossed the age of one year. Field teams supervised iron therapy for 2 months but did not make any attempt to document the compliance.

The enrolled children were supplemented for 6 months with a lipid-based nutritional supplement of vitamin B12 and/or folic acid. The supplement was prepared by NUTRISET, Ltd (MALAUNAY, France). Children were supplemented with a daily dose according to the age, 5 g (1 spoon) for children < 1 y and 10 g for children > 1 y. The supplement was administered by a fieldworker daily for 6 mo at home, except on Sundays and public holidays when the mother administered it herself. Each 10 g of supplement contained total energy of 54.1 kcal, 0.7 g proteins, and 3.3 g fat, 150 mg folic acid for the groups that were assigned to receive folic acid and 1.8 mg vitamin B12 for the groups assigned to vitamin B12. These doses were equivalent to 2 RDA for the respective age groups.

Biweekly home visits were conducted to ascertain information on morbidity. Weight was measured using digital Digitron scales with 50 g sensitivity (*Digitron, SCALES 'n' SENSORS INC., Delhi, India*), and length was measured using locally manufactured infantometer reading to the nearest 0.1 cm (*Nikhil traders, Delhi, India*). Anthropometric measurements were done at baseline as well as at the end of the study in all children.

2.3. Laboratory parameters

A blood specimen was obtained in EDTA-containing vacutainers (*BD, Franklin Lakes, NJ, USA*) for all children at baseline. A total of 272 children were assigned for blood sampling at the end of the study, equally distributed among the 4 treatments groups through a randomization process. Blood samples from 10 children could not be collected since the family refused or because they had left the area at the time of blood sample, leaving a total of 262 children with blood sample data at the end of the study. The blood specimen was centrifuged (*Remi Sales & Engineering LtD, Mumbai, India*) at approx. 450 × g at room temperature for 10 min in field settings. Plasma was separated and transferred into storage vials, and stored at −20 °C at the central laboratory until analysis. HemoCue AB (*HemoCue Hb Angelholm, Sweden*) was used to analyze Hb concentration [15,16]. Plasma concentrations of folate and vitamin B12 were estimated by microbiological assays [17,18], and plasma sTfR was analyzed using an immunoturbidimetric assay [19]. Plasma tHcy was analyzed using commercial kits (Abott Laboratories, Abott Park, IL, USA) [20].

2.4. Definitions

Anemia was defined as Hb concentration of <11 gm/dL and severe anemia as Hb <7 gm/dL on the basis of WHO criteria [21]. Iron deficiency was defined as sTfR concentrations >4.7 nmol/L [22]. We defined vitamin B_{12} deficiency as plasma vitamin B12 level of <200 pmol/L and folate deficiency as a plasma folate level of <7.5 nmol/L [12,23].

2.5. 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 committee 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. Plain-language statements explaining the study were provided to caregivers and written informed consent was obtained from the guardians of all child participants involved in the study. If no literate parent was present (i.e. the present parent was illiterate) at the time of consenting, a literate impartial witness signed the consent forms, witnessed the consenting process and that all parts of the consent form were explained to the parent(s) in detail.

2.6. Statistical methods

The forms used for data collection were designed in visual basic.net [24] and range and consistency checks were incorporated. Two data entry clerks entered and validated the data on the next day after the forms were filled. Queries were generated and resolved within 2 days and the data set completed within 3 days.

Continuous variables are given as means (SD), or medians (interquartile range) or means of log transformed values (SD). The skewed biomarker variables (plasma folate, vitamin B12, tHcy and sTfR concentrations) were log transformed to achieve normality. Categorical variables are presented as proportions.

We investigated the effect of folic acid and vitamin B12 supplementation given for 6 months on the concentration of hemoglobin and other biomarkers in multiple regression analyses. In these analyses each intervention group was compared to the placebo group for change in biomarker concentrations from baseline to end study.

We also compared these indices in children who were and were not anemic at baseline, i.e. between those who were treated with iron for two months and those who were not. We undertook these analyses with and without adjustment for relevant baseline

Please cite this article in press as: Kumar T, et al., Supplementation of vitamin B12 or folic acid on hemoglobin concentration in children 6–36 months of age: A randomized placebo controlled trial, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.07.002

characteristics: age, sex, height for age Z score, folic acid and vitamin B12 intervention and breastfeeding status. The statistical analyses were performed with Stata, version 13.1 (*StataCorp, College Station, TX, USA*).

3. Results

A total of 1000 children were enrolled and supplemented with placebo, folic acid, vitamin B12 or both for 6 months. Half of the children were supplemented with folic acid and another half were given vitamin B12 in a factorial design. These analyses are from children with blood samples both at baseline and at end of the study (n = 262). None with severe anemia (<7 g/dL) at baseline were included (Fig. 1). The baseline characteristics of the 262 children according to intervention groups are shown in Table 1. Mean age of enrolled children was 15.9 (7.3) mo and approximately 36% were infants. Median maternal education was 7.5 (3, 10) years and one fourth of the mothers were illiterate. More than one-third of the children were underweight, stunting was seen in approximately 43% of the children and around 11% were wasted (Table 1). About 70% children were anemic and received iron therapy for 2 months.

Table 2 shows biomarker concentrations at baseline and at study end. Mean hemoglobin concentration decreased in all of the study groups and the largest decrease was seen in children receiving folic acid. Folic acid and vitamin B12 supplementation improved folate and vitamin B12 biomarkers, respectively confirming our excellent compliance. The compliance is also reflected in the decrease in the concentration of tHcy at end of study where the effect was highest when vitamin B12 and folic acid were given together (Table 2). We also undertook these analyses adjusting for other baseline variables. None of the reported effects were altered when adjusting for these variables.

Six months supplementation of folic acid and/or vitamin B12 did not improve hemoglobin concentration or iron status. Indeed, folic acid supplementation tended to increase the risk of anemia as we observed a borderline significant reduction in hemoglobin concentration and iron status in children receiving folic acid (Table 3). Surprisingly, iron supplements for the initial 2 mo of the intervention period had limited effect on the Hb concentration at 6 mo, and almost 90% were still anemic at end of study among those who were anemic at baseline and to whom iron supplementation was provided (Table 4). Additionally 60% of those who were not anemic at baseline became so after 6 month. The decrease in Hb

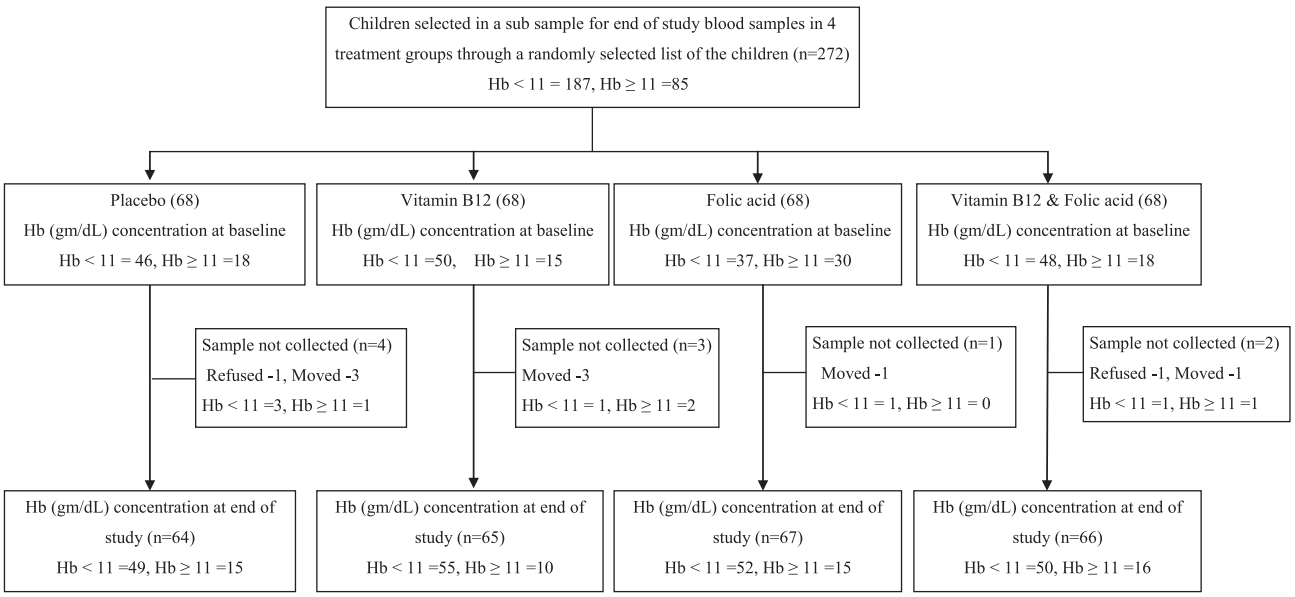

Fig. 1. Flow diagram for 272 children selected for the blood sampling at end of study in 4 treatment groups.

Table 1
Baseline characteristics of children aged 6–30 months for whom samples were collected at end study.

Characteristics	Placebo (n = 64)	Vitamin B12 (n = 65)	Folic acid (n = 67)	Vitamin B12 + Folic acid (n = 66)
Baseline				
Mean (SD) age (months) at enrollment	16.1 (7.2)	15.7 (7.2)	15.8 (7.5)	16 (7.4)
Age categories, n (%)				
<12 months	23 (35.9)	23 (35.4)	25 (37.3)	23 (34.9)
12 to 23 months	27 (42.2)	32 (49.2)	29 (43.3)	30 (45.5)
24 to 30 months	14 (21.9)	10 (15.4)	13 (19.4)	13 (19.7)
Boys, n (%)	33 (51.6)	25 (38.5)	30 (44.8)	37 (56.1)
Currently breast fed, n (%)	48 (75.0)	45 (69.2)	47 (70.2)	51 (77.3)
Literate mother, n (%)	51 (79.7)	45 (69.2)	53 (79.1)	49 (74.2)
Median (IQR) annual family income (INR*1000)	84 (48, 125.5)	60 (48, 120)	96 (66, 180)	72 (60, 120)
Mean (SD) weight (kg)	8.5 (1.4)	8.2 (1.6)	8.7 (1.6)	8.4 (1.7)
Mean (SD) length (cm)	74.3 (7.2)	73.3 (7.4)	73.8 (7.7)	74.0 (7.7)
Wasted (<-2 WHZ), n (%)	8 (12.5)	8 (12.3)	7 (10.5)	5 (7.6)
Stunted (<-2 HA Z), n (%)	27 (42.2)	26 (40.0)	30 (44.8)	29 (43.9)
Underweight (<-2 WAZ), n (%)	22 (34.4)	25 (38.5)	19 (28.4)	21 (31.8)

Please cite this article in press as: Kumar T, et al., Supplementation of vitamin B12 or folic acid on hemoglobin concentration in children 6–36 months of age: A randomized placebo controlled trial, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.07.002

Table 2
Biomarker concentrations at Baseline and End of study in the enrolled children.

	Placebo (n = 64)	Vitamin B12 (n = 65)	Folic acid (n = 67)	Vitamin B12 + Folic acid (n = 66)
Baseline				
Mean (SD) Hemoglobin concentration (gm/dL)	10.1 ± (1.3)	9.8 ± (1.3)	10.4 ± (1.5)	10.2 ± (1.2)
Median (IQR) Plasma folate concentration (nmol/L)	11.7 (6.7, 21.0)	8.7 (6.2, 16.2)	9.4 (5.5, 19.2)	8.3 (5.4, 16.7)
Median (IQR) Plasma vitamin B12 concentration (pmol/L)	250 (144, 355)	227 (165, 357)	246 (160, 387)	262 (182, 348)
Median (IQR) Plasma tHcy concentration[a] (μmol/L)	11.4 (8.9, 19.7)	11.7 (9.3, 13.6)	11.0 (9.0, 13.0)	12.7 (9.1, 17.0)
Median (IQR) Plasma sTfR (nmol/L)	3.5 (2.5, 4.7)	3.4 (2.5, 5.3)	3.5 (2.1, 4.3)	3.7 (2.2, 5.6)
End of study				
Mean (SD) Hemoglobin concentration (gm/dL)	9.9 ± (1.4)	9.6 ± (1.3)	9.7 ± (1.7)	9.7 ± (1.6)
Median (IQR) Plasma folate concentration (nmol/L)	15.3 (9.8–21.5)	13.3 (8.5–18.0)	46.5 (27.9–64.3)	47.7 (30.7–67.0)
Median (IQR) Plasma vitamin B12 concentration (pmol/L)	318 (191–404)	381 (282–567)	317 (248–492)	455 (307–605)
Median (IQR) Plasma tHcy concentration[a] (μmol/L)	10.7 (8.5–13.9)	8.5 (6.8–10.5)	7.3 (6.1–9.6)	6.8 (5.7–9.4)
Median (IQR) Plasma sTfR (nmol/L)	4.2 (3.1, 6.5)	4.1 (2.8, 5.9)	4.0 (2.6, 6.1)	3.6 (2.7, 5.6)

[a] n for tHcy are 66 and 65 in group 3 and 4 respectively at baseline and 63, 63, 66 and 64 respectively for the groups 1, 2, 3, and 4 at end study.

Table 3
Change in biomarker concentrations in three intervention groups compared to placebo from baseline to end of study in 6–30 months old North Indian children.

	Placebo (n = 64)	Vitamin B12 (n = 65)	Folic acid (n = 67)	Vitamin B12 + Folic acid (n = 66)
Hemoglobin concentration (gm/dL)	ref	−0.01 (−0.48, 0.45)	−0.42 (−0.88, 0.04)	−0.21 (−0.67, 0.25)
Plasma folate concentration (nmol/L)[a]	ref	−0.003 (−0.30, 0.30)	1.09 (0.80, 1.40)	1.20 (0.90, 1.49)
Plasma vitamin B12 concentration (pmol/L)[a]	ref	0.34 (0.17, 0.50)	0.19 (0.02, 0.36)	0.35 (0.18, 0.52)
Plasma tHcy concentration (μmol/L)[a],[b]	ref	−0.18 (−0.29, −0.07)	−0.24 (−0.35, −0.13)	−0.44 (−0.55, −0.34)
Plasma sTfR (nmol/L)[a]	ref	−0.07 (−0.24, 0.10)	0.04 (−0.13, 0.21)	−0.06 (−0.23, 0.11)

[a] Log transformed variables.
[b] n for tHcy are 66 and 65 in group 3 and 4 respectively at baseline and 63, 63, 66 and 64 respectively for the groups 1, 2, 3, and 4 at end study.

Table 4
Change in hemoglobin and sTfR concentration in anemic (who were given iron for 2 months) and non-anemic 6–30 months old children in North India.

Biomarkers	Not anemic (Hb ≥ 11) (n = 81)	Anemic (Hb < 11) (n = 181)	
Hemoglobin (g/dL)	Mean (SD)	Mean (SD)	Mean difference (95% CI)
Baseline	11.61 (0.65)	9.46 (1.02)	−2.16 (−2.4, −1.91)
End study	10.58 (1.32)	9.35 (1.44)	−1.24 (−1.61, −0.87)
Change from baseline to endofstudy	−1.03 (1.22)	−0.11 (1.29)	0.92 (0.58, 1.25)
Adjusted [1]			0.86 (0.52, 1.19)
Anemia at end study	47 (58%)	159 (88%)	
Serum Transferrin receptor (sTfR) (nmol/L)	Median (IQR)	Median (IQR)	
Baseline	2.9 (2.0–3.9)	3.9 (2.7–5.7)	
End study	3.3 (2.5–4.6)	4.3 (2.9–6.8)	
Log serum Transferrin receptor (sTfR) (nmol/L)	Mean (SD)	Mean (SD)	Mean difference (95% CI)
Change in concentration from baseline to end of study	0.27 (0.49)	0.12 (0.48)	−0.15 (−0.27, −0.02)
Adjusted[a]			−0.10 (−22, 0.03)

[a] Adjusted for age, sex, HAZ, breast feeding status and intervention groups folic acid and B12.

concentration from baseline to end of study was higher in the children with normal Hb at baseline [Mean (SD): 11.61 (0.65) to 10.58 (1.32)] in comparison to the anemic children [Mean (SD): 9.46 (1.02) to 9.35 (1.44)] where iron was given for 2 months (Table 4). Despite 2 months of iron supplementation, the change in iron status was not substantially different between the group receiving iron and the other children (Table 4).

4. Discussion

Vitamin B12 and/or folic acid supplementation did not have an impact on the hemoglobin concentration when given to children daily for six months. In fact, the prevalence of anemia increased during the 6 months of follow up in all four study groups. Iron, folate and vitamin B12 were the main predictors for Hb concentration at baseline [11] and Iron status decreased from baseline to study end. Iron status was the main predictor of anemia at baseline and children who received iron had a lower drop in Hb concentration during the observation period. Iron was given for a relatively short period, and 6 months after start of iron therapy almost 90% of the iron treated children were still anemic. The substantial increase in the concentration of both the vitamins and the decrease in tHcy reflect good compliance and biological effect of the vitamins provided.

The strengths of the study are that it is a carefully conducted randomized controlled trial with very low attrition and very high compliance [12]. An important limitation to the study was that it was not designed primarily to measure an effect on Hb concentration or on the risk of anemia, as the primary objective of the study was the effect on acute infections [12]. Furthermore there are multiple risks for anemia including iron deficiency, treating the children with folic acid and/or vitamin B12 without preventing other limiting factors might have underestimated the efficacy of these vitamins on the Hb concentration.

There are several causes of anemia including poor nutrition and chronic inflammation. The study could have benefitted from

Please cite this article in press as: Kumar T, et al., Supplementation of vitamin B12 or folic acid on hemoglobin concentration in children 6–36 months of age: A randomized placebo controlled trial, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.07.002

measuring markers of inflammation such as CRP or alpha-1-acid glycoprotein concentrations. With this information we could have estimated the contribution of inflammation on anemia. It should be noted that ongoing infection was an exclusion criteria and that the effect of inflammation is most likely limited.

The limited impact of iron treatment is indeed a compelling finding, which also could be explained by the presence of other limiting factors in addition to suboptimal compliance, with regard to the iron therapy, and absorption. In this study, we were only able to measure iron status and Hb concentration four months after end of iron treatment. It would also have been useful to know the effect of iron treatment immediately after end of iron supplementation. Furthermore, it should also be noted that there was no randomized comparison for iron supplementation as iron was given to all anemic children.

The results evolved in this analysis are contrary to the findings from our previous published paper where both vitamin B12 and folate status predicted Hb and anemia [11]. Although the main predictor for anemia and Hb was iron deficiency, iron treatment for a relatively short period did not suffice to prevent anemia after six months. This is an indication that high doses of iron for a short duration in anemic children may not work properly. The extent to which treatment with other doses and different durations is more feasible should be a prioritized research question. The dose used in our study might, in fact, have been too high and it is possible that a high dose of oral iron downregulates iron absorption that again renders a child at an even a higher risk of iron deficiency once supplementation ends. Strategies to prevent anemia should also be identified as 60% of those who were not-anemic became so after 6 mo of observation where only the vitamin paste or placebo paste were given and during a period when we attempted to ensure optimal treatment for all common illnesses.

The concentration of vitamin B12 was significantly higher in the children who were randomized to receive folic acid. It may be possible that folates play a role in the absorption or metabolism of vitamin B12. It might also be possible that folic acid supplementation increase bacterial growth and that these bacteria again produce vitamin B12. However, this observed effect might as well be a chance finding.

The use of folic acid alone or in combination with iron to combat anemia is common globally but our findings did not show any role of folic acid in anemia. However, this was not a primary objective of the study and study was not designed in such a way that we could argue strongly with sufficient evidence that use of folic acid alone or in combination with iron does not overcome anemia in children. It has also been observed that folic acid may impair cytotoxic immune responses, increase the risk of cancer and interfere with normal folate metabolism in the body [25–27]. So folic acid should be prescribed with caution and more information is needed regarding the effect of folic acid supplementation.

The end of study biomarker assessment was done in a subsample only as we did not have the necessary clearances to draw blood from all children at study end. The power to detect a difference of 0.31 g/dL units in hemoglobin concentration was as low as 45% (with the current sample size and SD of 1.4). However, the power to detect a difference of 0.5 g/dL (0.5 units in hemoglobin concentration, a clinically relevant difference) was more than 80% which is considered sufficient.

Iron deficiency explain most of the anemia prevalence in this population and other populations [1,7,11,28–32], so to reduce the prevalence of anemia in childhood iron should be given [33]. Our findings do not argue for widespread folic acid and vitamin B12 supplementation to combat anemia. Folic acid is often given with iron to treat or prevent anemia in young children. However, the role of these nutrients in anemia, particularly optimal dose and duration of iron supplementation should be a prioritized research question.

Source of support

Thrasher Research Fund (Project 9144) (USA) and Research Council of Norway (Norway) (Project no. 172226).

Authors' information and contributions

ST, TAS, NB designed research.
ST, TK, FA and SM conducted research.
CSY was involved in analysis of serum specimens.
BK was responsible for data management and data analysis.
TK, ST, BK, HPSS, HR and TAS analyzed the data and prepared the manuscript. TAS had primary responsibility for final content.

Conflict of interest

All authors read and approved the final manuscript and there was no conflict of interest among the authors, and with the funding agencies.

Acknowledgments

We acknowledge the input from Professor Halvor Sommerfelt and Mari Skar Manger at Centre for International Health, University of Bergen, Norway as well as from Gagandeep Kang, The Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences and Professor Ratnasamy Selvakumar, Department of Biochemistry from Christian Medical College, Vellore, India, and also from Dattatray S. Bhat from Diabetes Unit, King Edward Memorial Hospital, Rasta Peth, Pune for biochemical analysis. We also gratefully acknowledge the subjects and their families for their participation in the study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2016.07.002.

References

[1] Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet 2011;378(9809):2123–35.
[2] National family health survey (NFHS-3), 2005–06. Mumbai, India: International Institute for Population Sciences (IIPS) and Macro International; 2007.
[3] Venkateshwari A, Srimanjari K, Nallari P, Jyothi A. Iron deficiency anemia among the children in India—an overview. Available at: http://www.mecon.nomadit.co.uk/pub/conference_epaper_download.php5?PaperID=11875&MI%20METype=application/pdf. accessed April 2013.
[4] National family health survey (NFHS-2), 1998–1999. Mumbai, India: International Institute for Population Sciences (IIPS) and Macro International; 2000.
[5] Seshadri S. A database on iron deficiency anaemia (IDA) in India: prevalence, causes, consequences and strategies for prevention. India: Department of Foods and Nutrition. WHO Collaborating Centre for Nutrition Research. The Maharaja Sayajirao University of Baroda,Vadodara; 1999.
[6] Ministry of Health and Family Welfare (MOHFW). National consultation on control of nutritional anaemia in India. New Delhi (India): MOHFW; 1998.
[7] Stoltzfus R. Defining iron-deficiency anemia in public health terms: a time for reflection. J Nutr 2001;131(2S-2):565S–7S.
[8] Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, et al. Severe anemia in Malawian children. N. Engl J Med 2008;358(9):888–99.
[9] Schneider JM, Fujii ML, Lamp CL, Lönnerdal B, Dewey KG, Zidenberg-Cherr S. Anemia, iron deficiency, and iron deficiency anemia in 12–36-mo-old children from low-income families. Am J Clin Nutr 2005;82(6):1269–75.
[10] Duque X, Flores-Hernández S, Flores-Huerta S, Méndez-Ramírez I, Muñoz S, Turnbull B, et al. Prevalence of anemia and deficiency of iron, folic acid, and zinc in children younger than 2 years of age who use the health services

provided by the Mexican Social Security Institute. BMC Public Health 2007;7:345.
[11] Kumar T, Taneja S, Yajnik CS, Bhandari N, Strand TA, Mahesh M, et al. Prevalence and predictors of anemia in a population of North Indian children. Nutrition 2014;30(5):531–7.
[12] Taneja S, Strand TA, Kumar T, Mahesh M, Mohan S, Manger MS, et al. Folic acid and vitamin B-12 supplementation and common infections in 6– 30-mo-old children in India: a randomized placebo-controlled trial. Am J Clin Nutr 2013;98(3):731–7.
[13] Bhandari N, Bahl R, Taneja S, Strand T, Mølbak K, Ulvik RJ, et al. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. Pediatrics 2002;109(6):e86.
[14] Bhandari N, Bahl R, Taneja S, Strand T, Mølbak K, Ulvik RJ, et al. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomized controlled trial in an urban slum. BMJ 2002;324(7350):1358.
[15] Gehring H, Hornberger C, Dibbelt L, Roth-Isigkeit A, Gerlach K, Schumacher J, et al. Accuracy of point-of-care-testing (POCT) for determining hemoglobin concentrations. Acta Anaesthesiol Scand 2002;46(8):980–6.
[16] Bäck SE, Magnusson CG, Norlund LK, von Schenck HH, Menschik ME, Lindberg PE. Multiple-site analytic evaluation of a new portable analyzer, HemoCue Hb 201+, for point-of-care testing. Point Care 2004;3(2):60–5.
[17] O'broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathology 1992;45(4):344–7.
[18] Kelleher BP, Walshe KG, Scott JM, O'Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clin Chem 1987;33(1):52–4.
[19] Cotton F, Thiry P, Boeynaems J. Measurement of soluble transferrin receptor by immunoturbidimetry and immunonephelometry. Clin Biochem 2000;33(4):263–7.
[20] Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst (e) ine with the Abbott IMx analyzer. Clin Chem 1995;41(7):991–4.
[21] McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. Public Health Nutr 2009;12(04):444–54.
[22] Kolbe-Busch S, Lotz J, Hafner G, Blanckaert NJ, Claeys G, Togni G, et al. Multicenter evaluation of a fully mechanized soluble transferrin receptor assay on the Hitachi and cobas integra analyzers: the determination of reference ranges. Clin Chem Laboratory Med 2002;40(5):529–36.
[23] Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr 2007;86(5):1302–9.
[24] Visual Basic.net. Available at: http://vb.net-informations.com.
[25] Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136(1):189–94.
[26] Wien TN, Pike E, Wisløff T, Staff A, Smeland S, Klemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. BMJ Open 2012;2(1):e000653.
[27] Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomized trials: meta-analyses of data on 50 000 individuals. Lancet 2013;381(9871):1029–36.
[28] WHO/UNICEF. Focusing on anaemia: towards an integrated approach for effective anaemia control. Geneva (Switzerland): WHO; 2004. http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/en/index.html [accessed 22.10.12].
[29] WHO. Guideline: intermittent iron supplementation in preschool and school-age children. Geneva (Switzerland): WHO; 2011.
[30] Kotecha PV. Nutritional anemia in young children with focus on Asia and India. Indian J Community Med 2011;36(1):8–16.
[31] WHO/CDC. Worldwide prevalence of anaemia 1993-2005. WHO global database on anaemia. Geneva (Switzerland): WHO; 2008. http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596657/en/index.html [accessed 22.10.12].
[32] WHO. Iron deficiency anemia: assessment, prevention, and control: a guide for programme managers. Geneva (Switzerland): WHO; 2001.
[33] Sachdev HP, Gera T. Preventing childhood anemia in India: iron supplementation and beyond. Eur J Clin Nutr 2013;67(5):475–80.

Please cite this article in press as: Kumar T, et al., Supplementation of vitamin B12 or folic acid on hemoglobin concentration in children 6–36 months of age: A randomized placebo controlled trial, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.07.002