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Thalassemia carrier status and groundwater iron: Implication for iron supplementation program for children in Bangladesh

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Abstract

Background: Thalassemia, a congenital disorder of hemoglobin synthesis is characterized by low hemoglobin and high iron status, is prevalent in Bangladesh. Iron, consumed through drinking groundwater also increases the population iron status in Bangladesh. The study examined the effect of iron containing micronutrient powder (MNP) on the hemoglobin and ferritin status in Bangladeshi children with thalassemia and their non-thalassemia peers exposed to a high concentration of iron from drinking groundwater.

Design and methods: Three hundred twenty-seven children aged 2–5 years were recruited for an MNP efficacy trial. A sub sample ($n = 222$) were screened for thalassemia. Hemoglobin and ferritin levels were measured in children with and without thalassemia. Intake of iron from the key sources—diet, groundwater and MNP was measured. Mann Whitney and *t*-test were employed to compare the groups.

Results: Hemoglobin concentration of the children with thalassemia at the endpoint remained unchanged relative to the baseline; 11.56 ± 0.59 g/dL (Endpoint) versus 11.6 ± 0.54 g/dL (Baseline), $p = 0.83$. In children without thalassemia hemoglobin tended to increase; 12.54 ± 0.72 g/dL (Endpoint) versus 12.41 ± 0.72 g/dL (baseline), $p = 0.06$. Baseline reserve of body iron was significantly ($p = 0.03$) higher in thalassemia carriers (594 gm) compared to their non-carrier peers (558 gm). The increase of the infection-adjusted ferritin from baseline to the endpoint was 7.37% ($p = 0.7$) and 10.17% ($p = 0.009$) in the carrier and non-carrier groups respectively.

Conclusions: In Bangladesh, the coexistence of thalassemia and the exposure to a high concentration of iron from drinking groundwater renders anemia prevention program with a low iron MNP potentially lesser hazardous to the thalassemia carriers.

Keywords

Thalassemia, groundwater iron, anemia, Bangladesh

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Introduction

Thalassemia, a group of hereditary disorders resulting from genetic mutations involving hemoglobin synthesis, showing a wide range in severity from fetal death to mild anemia. The hallmark of thalassemia is the hyperplasia of the erythroid marrow and unproductive erythropoiesis affecting the tetramer structure of hemoglobin.[1,2] The disorder can lead to the secondary iron overload, defined as the iron overload which is not the result of direct mutations to proteins involved in iron absorption. Instead, it is a

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result of the inefficient erythropoiesis and a higher demand for and absorption of iron.[3] It has been reported that certain thalassemia carriers can have the iron absorption rates 3–4 times more than normal, increased serum ferritin levels, and decreased levels of hepcidin.[3,4] Hence, a few guidelines suggest the avoidance of routine supplementation of iron, unless there is a deficiency of iron.[5,6] However, in the developing country settings where dietary iron is sparse and predominantly plant-based, often a coexistence of thalassemia carriers and iron deficiency anemia is reported,[7,8] which warrants a correction by iron supplementation.

Bangladesh poses a different context. To control waterborne communicable diseases and the related mortalities and morbidities, the county had embarked upon the massive operation of utilization of groundwater in the 1970–80s; and since then, 97% of the rural population rely on groundwater for potable supply.[9] Iron concentration in ground water is high in many parts of the country.[9] In a nationally representative survey, a high level of iron in groundwater was independently associated with high iron status in children, adolescents, and women.[10] The similar observation was reported in another study.[11] Groundwater iron is bioavailable,[12] which may provide a wholesome supply of iron to the thalassemia carriers; and thus, may augment their iron stores. In this context, additional supplemental/fortified iron may not be required for erythropoiesis and could be counterproductive by loading of excess iron and the associated side-effects. Several small-scale studies suggest that the prevalence of the congenital hemoglobin disorders was variable by regions and the reported estimates were 2.9%–16%,[13] 17.2% in pregnant women,[14] 28% in non-pregnant women,[11] and 13.1% in children aged 2–5 years old.[15] A recent nationally representative survey reported the combined prevalence of hemoglobin E (ETT) and thalassemia B traits (BTT) was 11%.[16] Therefore, a fair proportion of the population is affected with the condition.

In the public health approach to manage population level anemia in association with thalassemia and/or the carrier states, there is no specific global guidelines taking into consideration the complex interplay of the conditions. The issue of groundwater iron in Bangladesh might have further compounded the scenario. The recent national guidelines for the control of anemia in Bangladesh[17] did not provide the directives as there is a lack of pertinent data.

Therefore, we conducted an exploratory study to examine the effect of supplementation of micronutrient powders (MNP) containing iron (12.5 or 5 mg iron) on hemoglobin and ferritin concentrations; and assess the iron-related side-effects in Bangladeshi children aged 2–5 years with or without thalassemia carrier state, who were exposed to a high concentration of iron from drinking groundwater.

Design and methods

The study was a nested sub study within a randomized controlled trial examining the effect of a low-iron MNP in preventing anemia and iron- related side-effects in Bangladeshi 2–5 years old children exposed to a high concentration of iron from drinking ground water. The trial received ethical approval from the Faculty of Biological Science, the University of Dhaka, Bangladesh (Ref# 46 / Biol. Scs. /2017–2018), and the Griffith University Human Ethics Committee, Australia (Ref# 2017/467). The trial was registered with the International Standard Randomized Controlled Trial Register, number ISRCTN60058115. Written informed consent was obtained from the parents allowing their children to take part in the study. Three hundred twenty-seven rural children who drank groundwater containing high level of iron (≥2 mg/L) were randomly allocated to receive the standard MNP (containing 12.5 mg of iron) or the low-iron MNP (containing 5 mg of iron); one sachet per day for 60 days. In a subsample of children ($n=222$), hemoglobin, ferritin, infection-biomarkers (CRP and AGP) were measured at baseline and at the end of intervention period.

We compared the groups, irrespective of the intervention (standard MNP and low-dose MNP), in regard to—hemoglobin and ferritin concentrations, prevalence of iron deficiency, episodes of iron related side-effects; and the intakes of iron from multiple sources such as groundwater, diet and the MNP supplements.

Sample size

The present study is nested in a larger study (i.e. RCT) examining the effect of a low-dose iron supplement on hemoglobin status in children from rural Bangladesh. Among the children selected for blood sample collection ($n=222$) ~13% ($n=29$) had thalassemia or Hemoglobin E disease carrier states, irrespective of the intervention groups. Daniel reported that for an exploratory/pilot study the minimum requirement of the cases is 20.[18] The cases of the thalassemia carriers of the present study ($n=29$) are higher than the requirement of an exploratory study. Furthermore, 30 samples would approximate a Gaussian distribution to provide a valid mean with standard deviation as per the central limit theorem.[19] Hence, the number of the thalassemia cases roughly conforms to the standard.

Data collection

At baseline and the endpoint, the intake of water was assessed by a 24-h recall method by using six time-prompts[11,15] and the intake of iron from water was estimated by multiplying the concentration of iron and the volume of water drunk over the preceding 24 h. Dietary intake of iron was assessed by a validated 7-day semi-quantitative food

Table 1. Selected participant and household characteristics by the thalassemia carrier status.

Variables	With thalassemia	Without thalassemia	*p*-value
	N = 222		
Thalassemia carrier status (%); *n*	(13.1); *n* = 29	(86.9); *n* = 193	<0.001
Age (month)	41.13 ± 9.9	39.81 ± 8.9	0.38*
Household expenses on food (BDT. per week)	1473 ± 548	1804 ± 878	0.06*
Prevalence of ID† (%); *n*			
Baseline	0.0; *n* = 0	2.07; *n* = 5	-
End-point	0.0; *n* = 0	0.0; *n* = 184	-
Intake of iron from groundwater‡ (mg/day); mean ±SD, *n*			
Baseline	4.91 ± 6.1, 29	6.08 ± 6.2, 193	0.17*
End-point	3.45 ± 3.41, 28	4.70 ± 4.85, 186	0.09*
Dietary intake of iron (mg/day), mean ±SD, *n*			
Baseline	3.04 ± 1.32, 29	3.14 ± 1.45, 193	0.96*
End-point	2.60 ± 1.02, 29	3.27 ± 1.78, 193	0.10*
Intake of iron from MNPs§	437.58 ± 214.75, 29	440.61 ± 228.17, 193	0.94*

*Mann Whitney Test.
†ID was defined as the infection-adjusted ferritin <12 ng/mL.[21]
‡Intake of iron from groundwater was calculated by multiplying the amount of water taken over the preceding 24 h and the concentration of iron in groundwater.
§Intake of iron was from both the MNPs as per random allocation of the treatment.

frequency questionnaire. Details of the methodology are provided in the parent trial.[15]

Biochemical analysis

After drawing a venous blood sample, hemoglobin was measured immediately using a hemocue photometer (Hemocue 301, Angleholm, Sweden). Ferritin was measured by an automated immunoassay analyzer (Cobas C311; Roche Diagnostics, Mannheim, Germany). Ferritin values were adjusted for infection for the raised values of CRP (>5 mg/L) and AGP (>1 g/L) by applying the Thurnham's principle.[20] Thalassemia carrier state was assessed by capillary zone electrophoresis of Hb at pH 9.4 (Capillary 2 system; Sebia, Evry, France). Concentration of iron in groundwater was measured by a hand-held portable colorimeter (HI-721; Hanna Instruments, USA) at baseline.

Statistical analysis

Data were first assessed for normality by histogram and the Shapiro-Wilk test. Since the data on the participant and household characteristics for example, age of the children, household expenses on food, intake of iron from various sources such as diet, groundwater and MNP were positively skewed, non-parametric test (Mann-Whitney *U* test) was used to compare between the children with thalassemia and children without thalassemia. Distribution of hemoglobin was largely bell-shaped by histogram and non-significant by the Shapiro Wilk test (results not shown). Therefore, the group comparison was done by the Independent Sample *t*-tests—that is, (a) between the children with thalassemia versus children without thalassemia and (b) between baseline and endpoint sorted by the presence or absence of the carrier children.

Regarding the unadjusted and infection-adjusted ferritin, the distribution of the data were positively skewed (significant results of the Shapiro Wilk test; results not shown). The ferritin values were first log-transformed. This improved the appearance of the normality curve consistent with the "bell shape" on histogram. We did *t*-test with unequal sample option on the log-transformed ferritin data; and back transformed to report the geometric mean of ferritin sorted by the groups and between baseline and endpoint. The statistical significance for the difference in ferritin between the groups and between the study points was tested by the Mann Whitney test. For all analyses, the p-value < 0.05 was considered statistically significant.

Results

Table 1 shows the selected characteristics of the participants by thalassemia status. The proportion of the children who were thalassemia carriers and non-carriers was 13.1% and 86.9% respectively ($p < 0.001$). Age of the participants was 41.13 ± 9.9 months and 39.81 ± 8.9 months in children with and without thalassemia respectively. Household expenses on the weekly food purchases did not differ between the groups ($p = 0.06$). Among the children with thalassemia, none had iron deficiency (ID) at baseline, whereas 2.07% had ID among their peers without thalassemia. None of the children had ID in either of the groups at endpoint. Intake of iron from groundwater

apparently was higher in the non-carriers both at baseline and endpoints, albeit with a non-significant statistical difference. Dietary intake of iron was similar between the groups at baseline; however, at the endpoint the children with thalassemia consumed apparently lesser than their non-carrier peers. The difference was not statistically significant ($p=0.10$). Over the intervention period, the total intake of iron from MNPs were 437.58 ± 214.75 mg and 440.61 ± 228.17 mg in the respective groups ($p=0.94$).

Figure 1 depicts the effect of iron supplementation (i.e. MNPs) on hemoglobin and ferritin concentrations in children with thalassemia compared with the children without thalassemia. Concentration of hemoglobin remained largely unchanged in the children with thalassemia over the intervention (11.60 ± 0.58 g/dL; baseline vs 11.56 ± 0.60 g/dL; endpoint). In the children without thalassemia an increasing trend in hemoglobin concentration was observed (12.41 ± 0.72 g/dL vs 12.54 ± 0.72 g/dL; $p=0.06$). The infection-unadjusted ferritin marked a statistically non-significant increasing trend in children with thalassemia showing 4.4% increase at endpoint. There was a smaller increment of 1.76% of the infection-unadjusted ferritin (statistically non-significant) at endpoint in children without thalassemia. The infection-adjusted concentration of ferritin showed increasing trend (non-significant) in children with thalassemia (7.37%). However, in their non-carrier peers the increase of ferritin was higher (10.17%) which was statistically significant ($p=0.009$).

Figure 2 shows the episodes of the iron-related intestinal side-effects over the 2 month course of the intervention in children with and without thalassemia receiving the MNP supplements. In children with thalassemia, the mean number of the episodes of loose stool was 3.5 and 0.6 in the subjects receiving the standard MNP and the low-iron MNP respectively ($p=0.10$). The mean of the episodes of diarrhea was 0.43 and 0.20 ($p=0.39$), respectively. In children without thalassemia, there were 1.86 and 1.4 episodes of loose stools in the subjects receiving the standard MNP and the low-iron MNP respectively ($p=0.42$).

Discussion

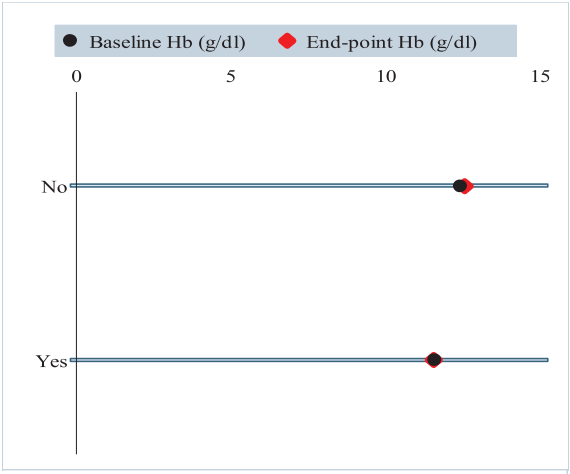
We assessed the changes in hemoglobin and ferritin concentrations (unadjusted and infection-adjusted) in Bangladeshi children with and without thalassemia carrier status after receiving MNPs containing iron (one sachet per day) for 2 months, exposed to a high concentration of iron from drinking groundwater.

The concentration of hemoglobin in children with thalassemia was statistically significantly lower than in the children without thalassemia both at baseline and endpoints. The hemoglobin concentration of the children with thalassemia at the endpoint remained unchanged relative to the baseline value, while that in the children without thalassemia tended to increase. However, there was no difference in the intake of iron from MNPs between the groups. The relative static level of hemoglobin despite a fair degree of supplementation of iron is consistent with the thalassemia carrier states which usually maintain a low level of hemoglobin.[5]

The mean concentrations of infection unadjusted ferritin at baseline were not statistically different between the groups. Albeit the concentration of ferritin was high relative to a less-diversified, predominantly cereal based rural diet in the country[22]; and considering a low level of infectious burden (% $\uparrow$CRP $<$ 10%; results not shown). The relative high values of the baseline ferritin possibly accounted for a high concentration of iron in groundwater which is the potable supply to the children. Over the 2 month long MNP intervention, the ferritin concentration remained nearly static, showing just 1.76% increase in children without thalassemia. On the other hand, this rate of increase was slightly higher (4.4%) in their thalassemia carrier peers, however the increase was not statistically significant. This observation is consistent with a predisposition of increased absorption of iron in thalassemia carriers when iron supplementation is provided.[5,6]

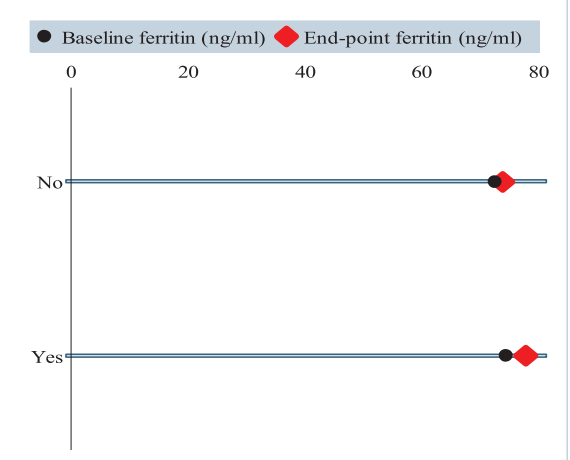
For the infection-adjusted ferritin, the children with thalassemia tended to have a slightly higher concentration at baseline than in the children without thalassemia (non-significant). However, at the endpoint, the increase (7.37%) of ferritin was non-significant in the carrier children, while the non-carrier peers registered a significant increase (10.17%; $p=0.009$). Yet the children without thalassemia tended to have a lower ferritin than in the children with thalassemia (non-significant). The reason for the significant rise in the infection-adjusted ferritin in children without thalassemia and non-significant increase in the thalassemia group is difficult to explain. It can be speculated that in this setting the thalassemia carriers are in a hyperferritinemia state both due to the medical condition and the groundwater iron. As such they are likely to uptake supplemental iron (e.g. through MNP) less efficiently compared to their non-carrier peers. Complementing this, the total reserve of body iron at baseline was higher in the children with thalassemia compared to the non-carriers (median: 594 gm vs 558 gm; $p=0.03$, results not shown). At the endpoint, the increment of the reserve iron was 3% and 7% respectively in the thalassemia-carrier and the thalassemia non-carrier groups implying a less efficient uptake of the supplemental iron in the thalassemia-carrier children. This observation complements the apparent higher episodes of loose stools in the carrier children which might happen when the absorption of supplemental iron is less efficient with a likelihood of the iron remaining unabsorbed in the intestines. This might potentially affect the composition of the gut microbiome adversely which renders the iron-associated side effects.[23,24]

A limitation of the study is the sample size of the thalassemia carrier group, which was small; hence the statistical



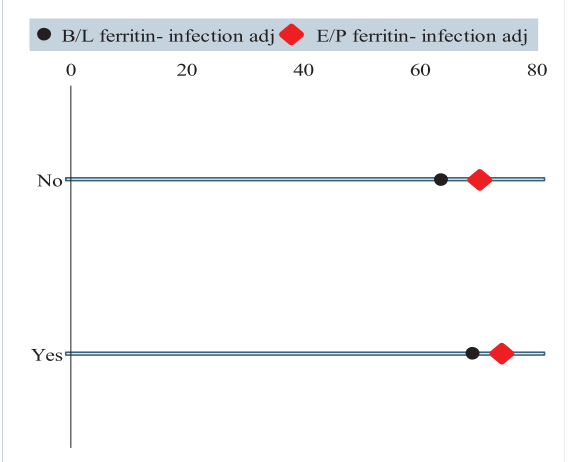
Legend: Yes=With thalassemia No=Without thalassemia-
B/L: Baseline; E/P: Endpoint

*†Hb (B/L): 11.6±0.54 [With thalassemia]
Hb (B/L): 12.41±0.72 [Without thalassemia]
†‡§Hb (E/P): 11.56±0.59 [With thalassemia]
¶Hb (E/P) 12.54±0.72 [Without thalassemia]
*Baseline difference between the groups (p<0.001)
‡Endpoint difference between the groups (p=0.005)
§Endpoint to baseline difference in Hb concentration in thalassemia carriers (p=0.83)
¶Endpoint to baseline difference in Hb concentration in normal children (p=0.06)
†Independent sample t-test



Legend: Yes=With thalassemia No=Without thalassemia
B/L: Baseline; E/P: Endpoint

*‡§Ferritin (B/L): 74.46±33.29 [With thalassemia]
§Ferritin (B/L): 72.57±45.70 [Without thalassemia]
†‡§¶Ferritin (E/P): 77.74±48.15 [With thalassemia]
‡§¶Ferritin (E/P): 73.86±35.16 [Without thalassemia]
*Baseline difference between the groups (p=0.44)
†Endpoint difference between the groups (p=0.81)
¶Endpoint to baseline difference of infection-unadjusted ferritin concentration in thalassemia carriers (p=0.96)
¶Endpoint to baseline difference of infection-unadjusted ferritin concentration in normal children (p=0.13)
‡Man Whitney U test between the groups or time points
§Ferritin data log transformed and back-transformed depicting the geometric mean



Legend: Yes=With thalassemia No= Without thalassemia
B/L: Baseline; E/P: Endpoint

*‡§Ferritin infection adjusted (B/L): 69.01±29.64 [With thalassemia]
§Ferritin infection adjusted (B/L): 63.68±35.22 [Without thalassemia]
†‡§¶Ferritin infection adjusted (E/P): 74.03*±36.65 [With thalassemia]
‡§¶Ferritin infection adjusted (E/P): 70.16**±32.27 [Without thalassemia]
*Baseline difference between the groups (p=0.24)
†Endpoint difference between the groups (p=0.52)
¶Endpoint to baseline difference of infection-adjusted ferritin concentration in thalassemia carriers (p=0.70)
¶Endpoint to baseline difference of infection-adjusted ferritin concentration in normal children (p=0.009)
‡ Man Whitney U test between the groups or time points
§Ferritin data log transformed and back-transformed depicting the geometric mean

Figure 1. Effect of MNP supplementation on hemoglobin and ferritin concentration by the thalassemia carrier states.

power was suboptimum to report the significant *p* values in relation to some of the presented estimates. However, the trend and direction of the estimates were expected as per our understanding of the theory and consistent over the assessments. Larger study is required to confirm this preliminary observation. Due to logistical constraints, gut

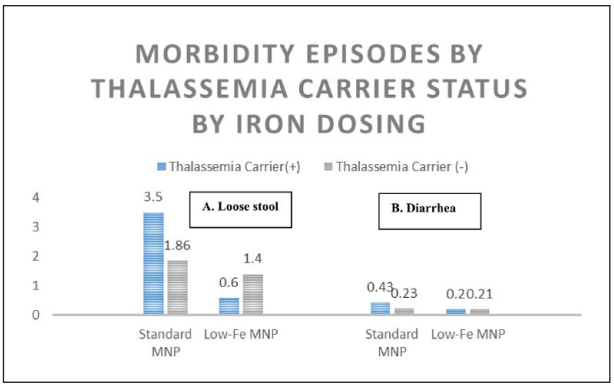


Figure 2. Iron-related side-effects by the thalassemia carrier status by iron dosing.

microbiome assessment was not done which could provide additional information on the microbiome composition; and thus, complementing its link with the differential iron reserve and absorption between the children with and without thalassemia. Hence, the lack of the gut microbiome assessment is a limitation. Strength of the study is that we have measured multiple sources of iron to account for the ferritin and hemoglobin status- for example, groundwater, dietary and MNP supplements.

In summary, the children with thalassemia had significantly lower hemoglobin concentrations than the children without thalassemia at both the assessment points. The infection-unadjusted ferritin showed a slightly higher predisposition for ferritin build up in the thalassemia carriers than in the non-carriers. This finding is consistent with Mehta and Pandya study.[25] The findings complement to some current guidelines, which does not recommend the routine iron supplementation in thalassemia carriers unless the ID coexists in them.[5,7]

There were hardly any cases with ID (infection-adjusted ferritin$<$12 ng/mL), unlike the observed co-existence of ID and thalassemia traits in other studies.[8,25] The lack of ID in the subjects can be plausibly explained by the iron from groundwater; and not accounted for dietary iron, which was suboptimum relative to the requirement in this age-group.[22]

In Bangladesh, there is a national policy for control of childhood anemia with the provision of the blanket iron supplementation in combination of other micronutrients that is, micronutrient powder (MNP) formulation containing 12.5 mg iron per dose. However, a significant proportion of the population is affected with thalassemia, mostly the carrier state; and as per some guidelines, these subjects are not recommended to receive the routine (i.e. unscreened) iron supplementation, as the risk of excess iron may increase. The risk is heightened in the background context of high level of groundwater iron in many parts of the country which contributes independently to a sufficient body iron status. In this particular scenario, supplemental iron at the current dose (i.e. 12.5 mg) in the subjects with thalassemia would likely to exacerbate the excess iron reserve and may increase the risk of the iron-related side-effects. Morbidity findings of the present study apparently complemented this.

In an ideal scenario, all children are required for screening for thalassemia. Following the screening, the body iron status (e.g. serum iron, serum ferritin) of the thalassemia carriers should be assessed[5,6] before deciding them to enroll for iron supplementation program. However, this is financially and logistically burdensome for a resource-poor setting like Bangladesh where the population size is enormous. Furthermore, operational challenge for the screening-based iron supplementation is massive in the country setting. Recently, a low-iron MNP containing a dose of 5 mg iron (instead of 12.5 mg iron of the standard MNP) has been shown to be efficacious in preventing low hemoglobin concentration and has shown a decreased incidence of the iron related side-effects, such as diarrhea, loose stool, nausea and fever; compared to the standard MNP in Bangladeshi children exposed to a high level of iron in groundwater.[15] In case the mass-screening for thalassemia and the assessment of iron status are infeasible, a reasonable option is to consider low iron formulation—for example, low-iron MNP (5 mg iron). This is likely to benefit the thalassemia carrier children residing among the overall population in two ways: firstly by reducing the risk of iron overload if not eliminating the possibility; and secondly, by decreasing the risk of the intestinal side effects.

In conclusion, in Bangladesh on the backdrop of the co-occurrence of thalassemia carrier states and the environmental exposure to a high concentration of iron from drinking groundwater; under a blanket iron supplementation policy for prevention of childhood anemia, a low iron MNP can potentially be less hazardous to the thalassemia carriers.

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Author's contribution

SR did the conceptualization, design, data collection, analysis, interpretation, and wrote the 1st draft of the manuscript. FA and PL revised the manuscript for important intellectual content and supported SR finalizing the manuscript—reviewing and editing. MRK supported the laboratory analysis and staff training.

Declaration of conflicting interests

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