Original Article

Improving the iron status of school children through a school noon meal programme with meals prepared using a multiple micronutrient–fortified salt in Tamil Nadu, India

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Background and Objectives: To improve the iron status of school children through noon meals prepared using a multiple micronutrient–fortified salt. **Methods and Study Design:** Children from a randomly selected school who consumed (intervention) and did not consume (reference) a noon meal prepared using a multiple micronutrient–fortified salt were studied over 1 year. A pre–post-test design for children aged 5–17years in reference (n=100) and intervention (n=128) groups was used. Levels of serum ferritin, soluble transferrin receptor (sTfR), alpha glycoprotein (AGP), and C-reactive protein (CRP) were assessed at baseline and at 1 year. In a subsample, urinary iodine was assessed. **Results:** sTfR decreased in the intervention group (−0.80 mg/L) but increased in the reference group (0.47 mg/L) at 1 year (p=0.0001).Body iron stores (BIS) increased in the intervention group (0.09 mg/kg body weight) and decreased (−0.58 mg/kg body weight) in the reference group at 1 year (p=0.028).These findings indicate an increase in iron deficiency in the reference group and a decrease in the intervention group. However, no changes in serum ferritin and urinary iodine were observed in either group or between groups. **Conclusions:** Iron status can be improved in schoolchildren in Tamil Nadu by increasing the amount of micronutrients in the fortified salt used for preparing noon-time school meals.

Key Words: multiple micronutrient–fortified salt, school meals, transferrin receptor, total body iron stores, iron deficiency

INTRODUCTION

Multiple micronutrient deficiencies are prevalent in children in India.[1,2] Many government programmes have been implemented to overcome this health concern, but they have not been successful. The government in Tamil Nadu, India, provides noon meals prepared using salt fortified with iron and iodine to children. However, anaemia levels in these children remain unacceptably high.[1] The iron content in salt supplied by the government is 1000 ppm, that is, 10mg in 10 g of salt. The amount of salt consumed by each child in one noon meal is approximately 2.5-3 g; thus, the iron intake of each child is only 2.5-3 mg per day. We hypothesise that this intake level is too low to have any effect on their iron status, which could contribute to the persistently high anaemia levels despite the use of double fortified salt in noon meals for the past several years. We therefore increased the micronutrient content in salt so that each child received at least 7.5–9 mg iron by consuming 2.5-3 g of salt from noon meals and analysed the iron and iodine status in the children after intervention with the multiple micronutrient-fortified salt. Thus, this study aimed to combat iron deficiency in school children in Chennai, TamilNadu, India, by providing noon meals prepared using multiple micronutrient- fortified powder salt enriched with iron, iodine, vitamin B-12, folic acid, and zinc, with higher micronutrient content.

METHODS

Study design

The study used a pre–post-test design to evaluate the efficacy of the multiple micronutrient–fortified salt in improving iron and iodine deficiencies.

Randomisation

The names of all schools in Saligramam, a suburb in Chennai that catered to children from families of lower socioeconomic status and that served government-provided noon meals were fed into a computer and the

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school randomly picked by the computer was chosen for the study.

Intervention and reference groups

Children who consumed the noon meal constituted the intervention group, and they consumed the fortified salt from September to August a study period of 1 year. Children who did not consume the noon meal and brought their own lunches from home formed the reference group. Blood samples were collected from all the participants at baseline and at the end of the study period. Urine samples were collected from a random subsample at baseline and at the end of the study. Serum ferritin, soluble transferrin receptor (sTfR), alpha glycoprotein (AGP), C-reactive protein (CRP), and urinary iodine were assessed. The study purpose was explained in detail to the noon meal coordinator and the school cooks, and the cooks were instructed to only use the fortified salt provided by us during the study period. The fortified salt was provided every month to the school. By using the amount of salt used in the kitchen for preparing noon meals, the total quantity of food prepared for the children, and the portion sizes served, the salt consumption of each child was calculated to be between 2.5 and 3 g per day. All the children in both the intervention and reference groups were periodically dewormed.

Inclusion criteria

This study included children aged 5–17 years after obtaining written informed consent from the head of the school and the parent or legal guardian of each child.

Exclusion criteria

This study excluded children whose parents did not provide informed written consent. Moreover, children with a haemoglobin level <8 g/dL (defined as severe anemia) were excluded from the study and were provided immediate medical intervention. Haemoglobin analysis was conducted only once before the start of the study to exclude anaemic children.

Ethical issues

This study protocol was in accordance with the Declaration of Helsinki, and all procedures involving human participants were approved by the institutional review board of our organisation.

Deworming

All the included children in both the intervention and reference groups were dewormed thrice at 6 monthly intervals during the study period by administering a tablet of albendazole (400 mg) at baseline, after 6 months, and 1 year after intervention. Deworming is performed to ensure elimination of any worms that may be competing for micronutrients and that the intestinal tract was clear for absorption of the micronutrients.[3,4]

Sample size

We considered a p level of 0.05 and power of 80% in a two-tailed test for all sample size calculations.

Manufacture of the fortified salt

A ribbon blender (Pragmatic Engineering, Chennai, India) was used at 50 rpm to manufacture the multiple micronutrient–fortified salt. The homogeneity of the salt's micronutrient content was established by testing the micronutrient content of the fortified salt in different parts of the blender. All micronutrients were found to be uniformly and homogeneously distributed in the fortified salt. The fortified salt was produced once in 4 months and was provided to the school once every month. The iron used was chelated ferrous sulphate owing to its enhanced bioavailability, and the remaining micronutrients were microencapsulated to prevent interaction among them as performed in our previous studies.[2,5-6]

Dosage of micronutrients

The fortified salt was used in preparation of the noon meal. Each child consumed approximately 2.5–3 g of salt per day. Ten grams of the fortified salt contained 30mg of chelated iron (3000 ppm), 900 µg of iodine (90 ppm), 12 µg of vitamin B-12, 300 µg of folic acid, and 30 mg of zinc. The calculated consumption amount accounted for approximately 100% of the required daily allowance of all micronutrients, except iron, for which it accounted for approximately 50% of the required daily allowance.

Blood collection and storage

Venous blood samples (5 mL) were drawn from the children at the school. The collected blood samples were allowed to clot, and then, serum was separated and transferred into vials. The vials were transported to the laboratory where the samples were frozen at −20°C within a few hours.

Laboratory analysis

Biochemical estimations of CRP, AGP, serum ferritin, sTfR, and urinary iodine were conducted. All the estimations, except that of urinary iodine, were performed in all children at baseline and at the end of the study. Urinary iodine was estimated in a random subsample of children at baseline and at the end of the study. Data of all the children were used for statistical analysis after correcting for inflammation.

Serum ferritin, sTfR, AGP, and CRP were determined using the sandwich ELISA method in a laboratory in Germany.[7] Dry ice was used during transportation of serum samples from India to Germany. The Sandell–Kolthoff reaction, as modified by Pino et al[8] was used for urinary iodine measurements. Children were defined to be iodine deficient if their urinary iodine was <100 mcg/L. Iron deficiency was defined as serum ferritin <15 µg/L or sTfR concentration >7.6 mg/L.[9] Body iron stores were estimated using the method reported by Cook et al.[10]

Validation of biochemical measurements

Urinary iodine estimations were conducted in duplicate in 10% of the samples. Serum ferritin, sTfR, CRP, and AGP were measured in duplicate for all the samples. The coefficient of variation was 3.01%, 4.58%, 6.55%, and 5.96% for ferritin, sTfR, CRP, and AGP, respectively.

Inflammation adjustments

Ferritin values increased with inflammation. According to their inflammation status, the children were grouped at baseline and at the end of the study as follows: reference group, in which the children showed no increase in acute phase proteins (CRP ≤5 mg/L and AGP ≤1 g/L) and ferritin, sTfR, and body iron stores values were used as obtained without any correction; incubation group, in which the children exhibited an increase only in CRP (CRP >5 mg/L) and normal AGP (AGP ≤1 g/L; all the ferritin values in this group were multiplied by a correction factor [CF] of 0.77); early convalescence group in which the children exhibited both increased CRP and AGP (CRP >5 mg/L and AGP >1 g/L; all ferritin values are multiplied by 0.53); and late convalescence group in which the children exhibited normal CRP (CRP ≤5 mg/L) and increased AGP (AGP>1 g/L; the ferritin values are multiplied by 0.75). To obtain the CF for sTfR, the children were grouped into the above-mentioned inflammation groups, and the geometric mean for each group was calculated. The CF for each group is calculated as follows: CF = Geometric mean of reference group/geometric mean of the respective inflammation group. The obtained CF is then multiplied to the values of the respective groups. The ferritin and sTfR values corrected for inflammation were used for calculating body iron stores.[11,12]

Statistical analysis

Statistical analysis was performed with SPSS 20.0 (SPSS Inc., Chicago IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle WA, USA). The efficacy of the intervention was compared between the intervention and reference groups. Thus, the efficacy of the multiple micronutrient–fortified salt in combating iron and iodine deficiencies was studied. Repeated-measures ANOVA was used to compare the effects of group x time for sTfR, ferritin, body iron stores, CRP, and AGP. If the interaction effect of group x time was significant ($p<0.05$), t tests between groups and paired t tests within groups were performed. Prevalence percentages for iron deficiency were compared using chi-squared tests. If data were not normally distributed, statistical analysis was performed after log transformation. Binary logistic regression was performed to compare the effects of group x time for the binary variable of iron deficiency. Significance was set at $p<0.05$. Urinary iodine was analysed using Mann–Whitney test between groups and Wilcoxon signed-rank test within each group. If a significance level of $p<0.05$ was obtained for differences in biochemical parameters at baseline between the intervention and reference groups, then ANCOVA was performed to adjust the baseline values to a common mean and then calculate the adjusted endpoint values.

RESULTS

A total of 128 children in the intervention group and 100 children in the reference group completed the study. Five children in the intervention group who were absent for more than 3 months during the study period and hence did not receive the intervention regularly were excluded from data analysis. Three children in the intervention group and four children in the reference group did not participate in the endline phlebotomy and hence were excluded from the study. Four children in the intervention group and three children in the reference group had haemoglobin levels <8 g/dL and were excluded from the study for treatment. This is shown in Figure 1.

Stability of the micronutrients in the fortified salt

All micronutrients were stable in the salt for 1 year (Table 1). Because the salt used was in powder form with very low concentrations of impurities, such as calcium and magnesium, and very low moisture content, the micronutrients were stable in the fortified salt for 1 year.

Age of the children

Children from class 1 to class 12 (age, 5–17 years old) who consumed the noon meal constituted the intervention group. Children who did not eat the noon meal and constituted the reference group were also from class 1 to class 12. No difference in the age of children was noted between the intervention and reference groups.

Iron deficiency

The sTfR decreased in the intervention group and increased in the reference group. Body iron stores increased in the intervention group and decreased in the reference group. These findings indicate a decrease in iron deficiency in the intervention group and increase in iron deficiency in the reference group over 1year.However, no improvement in serum ferritin was observed in the intervention group compared with the reference group during the study period.

Iron deficiency was analysed using binary logistic regression, and the results indicated that in the intervention group, the prevalence of iron deficiency reduced from 50% at baseline to 41.4% at the end of the study (Table 3), but this reduction was not significant. In the reference group, the iron deficiency was 34% at baseline and 35% at the end of the study, but the change was not significant.

The body iron stores and soluble transferrin receptor levels in the intervention and reference groups were significantly different at baseline. Therefore, ANCOVA was conducted in both groups to determine the effects starting from a common initial value. If the initial body iron store was 3.26 mg per kg body weight in both the intervention and reference groups, then the value would have increased to 3.31 mg per kg body weight in the intervention group and decreased to 2.72 mg per kg body weight in the reference group over the 1-year study period; this change is significant.

If the initial sTfR was 7.37 mg/L in both the intervention and reference groups, then the value would have reduced to 6.74 mg/L in the intervention group and increased to 7.63 mg/L in the reference group over the 1-year study period; this change is significant.

Therefore, ANCOVA results revealed a decrease in iron deficiency in the intervention group over the study period marked by a reduction in sTfR and an increase in BIS. The results also revealed an increase in iron deficiency in the reference group over the study period marked by a reduction in body iron stores and increase in soluble transferrin receptor levels.

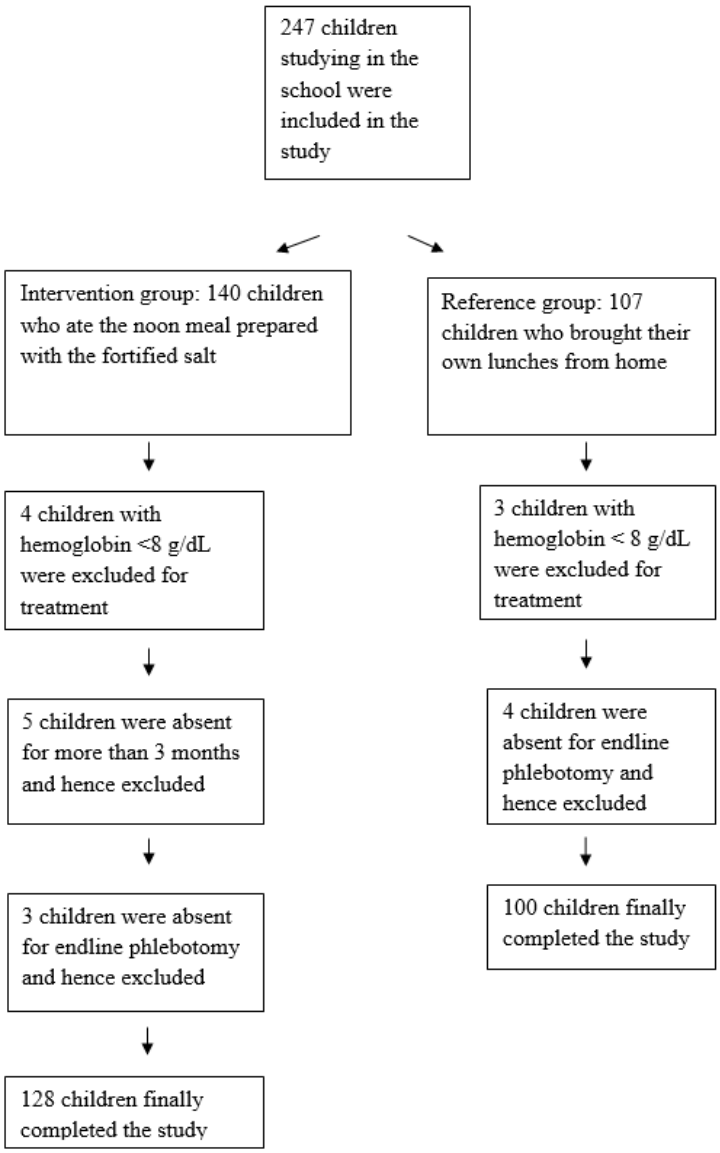

Figure 1. Flow diagram showing the number of children who started the study and those who completed it.

Table 1. Stability of the micronutrients in the multiple micronutrient–fortified powder salt during 1 year†

Nutrients	Label claim of the micronutrients in 10g salt	Initial levels on the date of manufacture	Levels after 6 months from the date of manufacture	Levels after 12 months from the date of manufacture
Iron (mg)	30	30.6	30.5	30.6
Iodine (µg)	900	960	946	906
Vitamin B-12 (µg)	12	13	12.6	12
Folic acid (µg)	300	350	325	310
Zinc (mg)	30	30.4	30.6	30.4

†Mean of the three batches prepared for the study.

CRP, AGP, and inflammation status

No significant difference in CRP was observed between the intervention and reference groups over the study period. The AGP decreased in the intervention group and increased in the reference group (Table 2). No increase in the acute phase proteins (CRP ≤5 mg/L and AGP ≤1 g/L) indicates no inflammation. With respect to inflammation, 78.2% of the children in the intervention group exhibited no inflammation at baseline, and 88% of the children exhibited no inflammation at the end of the study. In the reference group, 85% of the children exhibited no inflammation at baseline, and 76% of the children exhibited no inflammation at the end of the study.

Changes in urinary iodine

At baseline, the median urinary iodine in the intervention group was 165 µg/L and that in the reference group was 265 µg/L. The Mann–Whitney test results indicated a significant difference between the two groups. The results of the Wilcoxon signed-rank test revealed that the median urinary iodine nonsignificantly increased from 165 to 175 µg/L in the intervention group over the study period,

Table 2. Biochemical parameters, after correction for inflammation, in the two groups over a 1-year study period

Parameter	Intervention group: fortified salt					Reference group: no Intervention			
	N	Baseline	Post intervention	Change (post intervention - baseline)	ANOVA repeat measures (Intervention with reference) *p* value	N	Baseline	Post intervention	Change (post intervention - baseline)
Urinary iodine[†] (µg/L)	73	165 (100-270)	175 (110-317)	42 (-80-135)	0.132 [B]	44	265 (195-331)	210 (150-285)	-15 (-139-57)
Soluble transferrin receptor (mg/L)	128	7.90±3.53	7.10±3.25	-0.80±2.33	0.0001[A]	100	6.71±1.62	7.18±2.20	0.47±2.15
Ferritin[‡] (µg/L)	128	24.17±19.56	22.44±22.10	-0.2±14.26	0.625 [B]	100	28.13±21.28	25.34±24.50	-0.7±16.75
Body iron stores (mg/kg body weight)	128	2.84±3.68	2.93±3.91	0.09±1.69	0.028 [A]	100	3.79±2.83	3.21±3.64	-0.58±2.83
CRP (mg/L)	128	0.58±1.27	0.58±1.25	0.01±1.50	0.143 [B]	100	0.91±2.86	2.37±10.84	1.46±11.07
AGP (g/L)	128	0.81±0.26	0.74±0.29	-0.07±0.32	0.001 [A]	100	0.75±0.26	0.84±0.33	0.09±0.36

All data are presented as mean±SD unless otherwise indicated.
[†]Median (range 25th–75th percentile). Wilcoxon signed-ranks test and Mann–Whitney test.
[‡]Geometric mean±SD.
Mann–Whitney test: Intervention and reference, baseline p=0.001, endline p=0.132.
Wilcoxon-signed ranks test: Intervention p=0.165, reference p=0.150.
[A]Significant improvement.
[B] Nonsignificant changes.

Table 3. Prevalence percentage of iron deficiency in the intervention and reference groups at baseline and post intervention after correction for inflammation

	Intervention group- fortified salt			Reference group- No intervention			
	Sample size	Baseline	Post Intervention	Sample size	Baseline	Post Intervention	*p* value Binary logistic regression Group x time Interaction (Intervention group with reference group)
Iron deficiency prevalence (%)	128	50	41.4	100	34	35	0.253[A]

[A]Nonsignificant improvement.

whereas in the reference group, the median urinary iodine nonsignificantly decreased from 265 to 210 μg/L. The Mann–Whitney test results showed that there was no significant difference in the urinary iodine values between the intervention and reference groups at the end of the study.

DISCUSSION

For the past several years, the Government of India has been providing double-fortified salt for use in the preparation of noon meals in Tamil Nadu. Ten grams of the double-fortified salt contains 10 mg (1000 ppm) of iron and 300 μg (30 ppm) of iodine. The children in the study consumed approximately 2.5–3 g of salt per meal. This means that the children consumed approximately 2.5–3 mg of iron through the noon meal every day. We believe that this iron dose per child per day is too low to improve the iron levels in the children and mitigating iron deficiency. The salt was formulated for daily consumption in all the meals of the day by the general population; a daily consumption of approximately 10 g per person will result in an intake of 10 mg iron per person per day, which is approximately 50% of the required daily allowance. The required daily allowance of iron is approximately 22 mg iron per person per day. When this salt is used in the noon meal, each child consumes only 2.5–3 g, constituting an intake of only 2.5–3 mg of iron, which is only approximately 10% of the required daily allowance of iron. This intake is grossly insufficient to mitigate iron deficiency in these children.

In this study, we added three times the dose of micronutrients such that 10 g of the fortified salt contained 30 mg of iron, 900 μg of iodine, 30 mg of zinc, 12 μg of vitamin B-12, and 300 μg of folic acid. When the children consume 2.5–3 g of this fortified salt per day, they acquire approximately 7.5–9 mg of chelated iron, 225–270 μg of iodine, 7.5–9 mg of zinc, 3–3.6 μg of vitamin B-12, and 75–90 μg of folic acid. The required daily allowance for iron is 22 mg in children of this age group, and children in this study received approximately 50% of the required daily allowance through the fortified salt. The required daily allowance for zinc is approximately 10mg, and the children in this study received 75% to 90% of the required daily allowance through the fortified salt. The required daily allowance for vitamin B-12 is 1 μg and that for folic acid is 100 μg, and the children in this study received 100% of the required daily allowance for vitamin B-12 and 75%–90% of that for folic acid through consumption of this fortified salt through their noon meal.

Although in this study, three times the dose of micronutrients was added to the fortified salt, after 1 year, the ferritin levels did not increase significantly in the intervention group. The prevalence of iron deficiency nonsignificantly decreased in the intervention group from 50% at baseline to 41.4% at the end of the study. This may be attributed to the insufficient study period of 1 year or the requirement of a dose of more than 3 times that of the micronutrients. For example, increasing the dose to 4 times that of the micronutrients might improve the ferritin stores and significantly reduce iron deficiency in the intervention group.

The decrease in body iron stores and increase in soluble transferrin receptor levels in the reference group observed in this study have been reported in our earlier studies[5,13] and studies by others.[14] This may be because of poor availability of iron from the cereal diets of these children during the growth and development years during which iron is most needed.

When children experience protein energy malnutrition, serum ferritin has limited value in evaluating the iron status because protein energy malnutrition is often accompanied by inflammation and inflammation distorts ferritin values. In this study, the children did not show any signs of protein energy malnutrition. Moreover, protein energy malnutrition is characterised by high inflammation, causing an increase in acute phase proteins. Inflammation also increases ferritin levels. In this study, almost 80% of the children exhibited non-elevated levels of the acute phase proteins CRP and AGP, thus indicating that they had no inflammation. Furthermore, ferritin and sTfR were corrected for inflammation, and the corrected values were used to calculate body iron stores.

Zinc, in the presence of iron, reduces the bioavailability of iron. We have conducted studies using salt fortified with both iron and zinc[5] apart from other micronutrients as well as studies using salt fortified with iron and other micronutrients but not zinc.[13] The results of these studies revealed that the presence of zinc does not interfere with iron absorption, evidenced by a significant improvement in hemoglobin levels as well as body iron stores and reduction in soluble transferrin receptors and iron deficiency. Therefore, in all our later studies including the present study, we used the salt fortified with both zinc and iron.

Rice is the staple food of the studied children. It is known that phytates contained in rice bind with iron and zinc, which cause iron and zinc to not be absorbed and bioavailable and be eliminated in the feces. Our fortified salt contained chelated iron, which has higher bioavailability thereby making iron bioavailable despite the presence of phytates in the food. The use of chelated iron in the fortified salt may have contributed to the improvement in the body iron stores and reduction in soluble transferrin receptor in the intervention group.

As mentioned previously, we believe that the current Indian government policy of providing double-fortified salt for preparing the noon meal will not help alleviate anaemia. For an evident improvement, the fortified salt should have at least 30 mg of iron per 10 g of the fortified salt in contrast to the current 10 mg iron per 10 g of the fortified salt.

We added vitamin B-12 and folic acid apart from iron because both these vitamins are essential for erythropoiesis or red blood cell formation and hence act synergistically with iron to reduce iron deficiency. We also added zinc to the fortified salt because we have reported zinc deficiency extensively in school children in our earlier studies[5] and because zinc helps improve the immune system and reduces morbidity.

Conclusion

The multiple micronutrient–fortified salt with 3 times more iron than the iron present in double-fortified salt conventionally used by the Government of Tamil Nadu increased body iron stores and reduced soluble transferrin

receptor levels, thereby reducing iron deficiency in children who consumed meals prepared using this salt. However, ferritin stores could not be improved in the 1-year study period. Therefore, to significantly reduce iron deficiency in the studied children, it is necessary to use salt fortified with 3 times more iron to prepare the noon meals.

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AUTHOR DISCLOSURES

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