Original Article

An efficacy study on alleviating micronutrient deficiencies through a multiple micronutrient fortified salt in children in South India

Malavika Vinod Kumar PhD[1], Praveen K Nirmalan MPH[2], Juergen G Erhardt PhD[3], Lakshmi Rahmathullah MBBS, DTPH[4], Srinivasa Rajagopalan PhD[5]

[1]*Sundar Serendipity Foundation, Chennai, India*
[2]*Clinical Research Unit, Fernandez Hospital, Hyderabad, India*
[3]*VitMin Lab, Willstaett, Germany*
[4]*Family Health and Development Research Service Foundation, CCD Campus, Sevayur, India*
[5]*MS Swaminathan Research Foundation, Chennai, India*

Background: Multiple micronutrient deficiencies are prevalent in India. Objective: The study aims to establish the efficacy of multi-micronutrient fortified salt in addressing multiple micronutrient deficiencies among children compared to nutrition education and no intervention in Tamilnadu. Methods: The study employed a community based randomized controlled trial designed to study the impact of multiple micronutrient salt (micronutrient group) in comparison with nutrition education (education group) and no intervention (control group) on haemoglobin, serum ferritin, soluble transferrin receptor, body iron stores, serum retinol and urinary iodine outcomes over a period of 8 months. The fortified salt contained iron, iodine, vitamin A, vitamin B_{12} and folic acid. All the children were dewormed at baseline and at the end of the study just before the biochemical measurements. Results: There was a significant improvement in most biochemical parameters studied in the micronutrient group when compared with the control group whereas this was not seen between the education and control. Over 8 months, in the micronutrient group, hemoglobin increased by 0.52 g/dL, retinol by 8.56 μg/dL, ferritin by 10.8 μg/L, body iron stores by 1.27 mg and the decrease in the prevalence of retinol deficiency was from 51.6% to 28.1%, anaemia from 46.0% to 32.6%, iron deficiency from 66.9% to 51.3% and iron deficiency anaemia from 35.2% to 31.0%, while the prevalence of all these deficiencies increased or the changes were not significant in the other two groups. Conclusions: Multiple micronutrient fortified salt was able to improve iron and vitamin A status, whereas this was not seen in the nutrition education group.

Key Words: multiple micronutrient fortified salt, micronutrient deficiencies, biochemical assessment, children, India

INTRODUCTION

Multiple micronutrient deficiencies are prevalent in many developing countries including India. The national family health survey done by the Government of India in 2005-2006 showed that the prevalence of anaemia in children 6-35 months of age was 79.2%. This survey also showed that 56.2% of women, 57.9% of pregnant women and 24.3% of men were anaemic.[1] Anaemia is therefore a huge national problem. One of the reasons for anaemia is the consumption of plant-based cereal diets.[2,3] Dietary phytate inhibits the absorption of many micronutrients, notably iron and zinc. Another reason for anaemia is because there is also very little consumption of non-vegetarian food, due to its high cost; however meats have a high bioavailable haem iron and vitamin B_{12}. Among micronutrient deficiencies, iron and iodine deficiencies affect more than 30% of the global populations.[4] Earlier studies have reported multiple micronutrient fortification of beverages,[5-7] or biscuits.[8] Multiple micronutrient interventions in school children in India[9,10] have shown promising results. Our earlier studies in providing multiple micronutrients to school children have also shown that multiple micronutrient deficiencies can be successfully combated.[11,12] We have studied multiple micronutrient fortification of salt in earlier studies.[13-15] We have seen in our earlier study that the prevalence of serum retinol deficiency in children in Tamilnadu to be 57.1%.[14] The prevalence of angular stomatitis due to B complex deficiencies in our earlier study was 12.8%.[14] This only confirms that multiple micronutrient deficiencies exist in children in India and needs to be combated. In our study area, namely rural Tamilnadu, there have been no existing micronutrient interventions in children in the target age

Corresponding Author: Dr Malavika Vinod Kumar, Sundar Serendipity Foundation 6G Century Plaza, 560/562 Anna Salai, Teynampet, Chennai 600018, India.
Tel: +91 44 24349230; Fax: +91 44 24349352
Email: vinodkumar_m_k@hotmail.com
Manuscript received 8 March 2013. Initial review completed 16 August 2013. Revision accepted 25 November 2013.
doi: 10.6133/apjcn.2014.23.3.08

group of above 5 years, although the government provides vitamin A drops to children in the 6-36 months age group. For salt fortification to be successful, the micronutrients should not change the colour, odour, or taste of the food, should be stable at cooking temperatures, and should be bioavailable as was the case in our earlier studies with multi-micronutrient fortified salt. In this study, we have compared two different approaches to combat multiple micronutrient deficiencies, namely nutrition education (education group) and multiple micronutrient fortification of salt (micronutrient group) with the no intervention (control group). Nutrition education was included because other than supplementation and fortification, the other strategy used to combat micronutrient malnourishment is dietary diversification which can be brought about by nutrition education. We decided to add iron, iodine, vitamin B_{12}, folic acid and vitamin A to the multiple micronutrient fortified salt because these are the most common micronutrient deficiencies that are seen.

Aims/objectives

The study aimed to compare the efficacy of a multiple micronutrient fortified salt (micronutrient group) in addressing multiple micronutrient deficiencies among children compared to the efficacy of nutrition education (education group) and no intervention (control group).

MATERIALS AND METHODS

Study location

The project was carried out in Kariapatty block of Virudhnagar district in the state of Tamil Nadu in South India.

Study design

The study employed a community based randomized controlled trial designed to study the impact of multiple micronutrient salt (micronutrient group) in comparison with nutrition education (education group) and no intervention (control group) in combating multiple micronutrient deficiencies.

Randomization

The villages for the three arms of the trial were selected randomly from the villages in Kariapatty block of Virudhnagar district. Kariapatty block in Tamil Nadu (that has 144 villages) was considered as the sampling frame for the study. We used villages in this block as the primary unit of randomization. Thus, villages and all eligible children in these villages were randomized to receive any one of the three arms. The sample frame had villages that were quite large in population size and villages or hamlets that had a very small population. To ensure representativeness, we combined contiguous 'smaller' villages and divided 'larger' villages such that each village in the sample frame had a child population that ranged between 200-400 children. Villages in the sample frame were then arranged in alphabetic order. The villages were chosen using a simple random strategy without replacement from the sampling frame of Kariapatty block. We randomly selected three villages for the study-Salaimaraikulam, Aarasakulam, and Kallupatti using a computerized random number generator. After the random selection, we then verified whether the children in the three groups of the study were matched for age, and socioeconomic status. We also confirmed that the selected villages had equal access to primary healthcare centres. The main occupation of the villagers in the selected villages was agriculture and the average income of the families was about Rs 2000 (USD 40) per month. The consumption of iodised salt in the selected villages varied from 0% to 10% of the households.

Inclusion criteria

This study included children aged 5 to 15 years, after obtaining written informed consent from the parents of children or their legal guardians.

Exclusion criteria

This study excluded subjects whose parents or legal guardians did not provide informed consent. Subjects who had a haemoglobin level less than 8 g/dL (defined as severe anaemia) were excluded from the study to enable immediate medical intervention for them.

Ethical issues

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of Sundar Serendipity Foundation. Informed written consent was obtained from the parents or legal guardians of all the children. Verbal consent of the child was taken during phlebotomy. The parents of the children were informed that blood tests would be performed on all the children and those children with severe anaemia (haemoglobin <8 g/dL) would be treated with ferrous sulphate tablets and excluded from the study. Anaemic children at the end of the study in all the arms of the trial were treated with ferrous sulphate tablets (60 mg elemental iron) for a period of three months.

At the local administrative level, consent was sought and obtained from the district level education and health authorities, and block level education and health authorities. In the villages, we initially approached the village level administration and explained the study seeking consent. Then, we held up to three community level meetings in the selected villages explaining the study. We also contacted households in the village to explain about the study.

Deworming

Children in all the three arms of the study were given a tablet of albendazole (400 mg) at baseline and post intervention after 8 months. Deworming was done to ensure that there were no worms competing for the micronutrients and that the intestinal tract was clear for absorption of the micronutrients.[16,17]

Study intervention arms

This study had three arms-two intervention arms and a control arm, where there was no intervention. The first arm received the fortified salt (micronutrient group), the second arm received health education (education group), and the third arm had no intervention (control group). Baseline data collection and phlebotomy concluded by December 2008 and the study commenced from January 2009 until August 2009.

In the first arm (micronutrient group), the design of the study was explained to all the heads of the households and the women in the households, and they were then educated on the role of micronutrients in human health and about the necessity to use only the fortified salt provided for cooking all their meals for the next 8 months until the study concluded. All these households were provided with the multiple micronutrient fortified salt every month for a period of 8 months. From the size of the households we could calculate the average salt consumption per household theoretically and this quantity rounded off to the nearest kilogram was provided to the families every month. When the health workers visited the households every month, they collected the left-over packets of the fortified salt of the previous month. From this we calculated whether the families were using the fortified salt provided by us. It was found that all the families were using the fortified salt provided by us only.

In the second arm (education group), the households were divided into 4 groups, and each week, a group was covered for nutrition education. Prior to the start of the study, a list of locally available foods which are rich in micronutrients and simple, culturally acceptable recipes for regular use of these locally available foods was communicated. The nutrition education was given to all the women residing in the households, who were involved in cooking and providing food to the families. The study commenced in January 2009 and in the month of January 2009, the communities in this arm were given a general introduction of the role of micronutrients in human health and the importance of this nutrition education. Then, every month a specific topic was chosen and in-depth education was given on that topic. The topic for February 2009 was vitamins and their role in human health, the topic for March 2009 was iron, the topic for April 2009 was iodine and the topic for May 2009 was calcium. The nutrition education sessions involved the details about the prevalence of the micronutrient deficiency, and the functional consequences of the deficiency. In the subsequent months, a brush up of the above topics was given along with education about general health and hygiene and the importance of proper nutrition in child development. In the third arm, there was no intervention (the control group).

Sample size

We have considered α of 0.05 and power of 80% with a two-tailed test for all sample size calculations. Our earlier experiences with the use of fortified salt in children showed a mean increase in haemoglobin from 0.4 to 0.7 g/dL between the experimental and control groups, with a standard deviation of about 1 to 1.25.[13-15] If a similar increase is assumed in this study, a sample size of 154 children in each group is required. With respect to serum retinol, our earlier studies have seen a mean increase of 5.56 μg/dL, between the experimental and control groups, with a standard deviation of 12.9. If a similar increase is assumed in this study, a minimum sample size of 85 children in each group is required. From our analysis of urinary iodine in our earlier studies, we arrived at a minimum sample size of 45 children in each arm for urinary iodine analysis. Similarly, from our earlier study[14] and other studies[9] we arrived at a minimum sample size of 130 for soluble transferrin receptor (sTfR), serum ferritin, C-reactive protein (CRP) and α1-acid glycoprotein (AGP).

Manufacture of the fortified salt

The multiple-micronutrient fortified salt was manufactured in a ribbon blender (Pragmatic Engineering, Chennai, India) which had 50 rpm blend speed. The homogeneity of the salt's micronutrient content was established at the manufacturing stage by assessing the micronutrient content of the fortified salt in different parts of the blender. Six samples were taken from the 4 corners (samples A, B, C, D), and the centre from the top (sample E) of the blender and the 6th sample (sample F) was taken from the discharge unit at the bottom of the blender. It was determined that all of the micronutrients were uniformly and homogeneously distributed within the product. The method of manufacture was similar to the method used to manufacture multiple micronutrient fortified salt in our previous studies.[13-15] The salt was produced on a quarterly basis, and sent to the villages. In the villages the salt was distributed to the households every month.

When multiple micronutrients are used, they interact with each other leading to losses in potency. Iron is stable and best bioavailable in an acidic pH whereas vitamin A is unstable and loses its potency in an acidic environment. To prevent such interactions, the micronutrients are microencapsulated to prevent interactions with each other and they remain in an environment best suited for them. Vitamin B_{12} is magenta in colour and folic acid is turmeric yellow in colour, and so they are microencapsulated so that even when added to salt, the white colour of the salt is not altered by these coloured fortificants.

Dosage of micronutrients

The salt was used in all the meals prepared in the household. The children consumed three meals and an evening snack every day. It was found out that the consumption of salt ranged from 7.9 g for 5 years old children and 11.4 g in 15 years old children. This data was obtained from food frequency questionnaire administered to the parents and by using standardized measurement cups during the questioning. Therefore, the fortified salt was prepared such that 10 g of the fortified salt contained about 1-RDA of the micronutrients, except iron which given at a dosage of 10 mg per day (30% RDA) as the iron was chelated, instead of 28 mg iron which was the RDA.[18] Ten g of the fortified salt contained 3000 IU of vitamin A (from microencapsulated vitamin A acetate), 10 mg of chelated iron (from chelated ferrous sulphate), 40 ppm iodine (from microencapsulated potassium iodate), 1 mcg of vitamin B_{12} (from microencapsulated B_{12}) and 100 mcg of vitamin folic acid (from microencapsulated folic acid). Each micronutrient was separately microencapsulated in the same way as in our previous studies.[19]

Iron was chelated in the same way as in our previous studies.[19] The iodine source was potassium iodate and it was microencapsulated with cellulose acetate phthalate (GM Chemicals, Mumbai, India).

Blood collection and storage

Venous blood samples (5 mL) were drawn from each child at the schools and other central places in the villages

and 500 μL transferred into vials with ethylene diamine tetraacetate (EDTA) as an anticoagulant. The haemoglobin measurements were performed on these samples within a few hours of blood collection at the school in the villages. The remaining 4.5 mL of blood was transferred into vials covered with black paper to prevent exposure to light, and the blood was allowed to clot. The blood samples stored on ice were transferred to the laboratory at the end of every day. Serum separation was performed in the laboratory and the samples were frozen at -20°C at the end of the day when phlebotomy was conducted. In those children where only haemoglobin measurement was performed, only 0.5 mL of venous blood was drawn and transferred into vials with EDTA as an anticoagulant. During serum retinol estimations, the samples were processed in a dark room with yellow lighting to prevent retinol isomerization. SF, sTfR, CRP and AGP were done in a laboratory in Germany. The serum samples were transported on dry ice from India to Germany.

Laboratory analyses

The biochemical estimations done were for haemoglobin, serum ferritin, sTfR, CRP, AGP, serum retinol and urinary iodine. Haemoglobin was measured in all the children in all the three arms of the study (n=215 in arm 1, n=214 in arm 2 and n=217 in arm 3) twice during the study, at baseline and post intervention after 8 months. Serum retinol, serum ferritin, sTfR, CRP and AGP were done in a random subsample of children (n=154 in arm 1, n=157 in arm 2 and n=140 in arm 3) at baseline and post intervention. All these samples were used for statistical analysis after making corrections for inflammation. Urinary iodine was done in a random sub-sample (n=46 in arm 1, n=51 in arm 2 and n=54 in arm 3) at baseline and post intervention. Baseline and post intervention biomarker analysis was performed on the same random subsample of children.

Haemoglobin was estimated by the cyanmethemoglobin method with a Colorimeter.[20] Serum retinol was measured by a rapid, reverse-phase HPLC method (HPLC-Shimadzu, Japan). NIST serum sample (SRM 968c- lyophilized frozen serum sample with certified retinol values) was used to calculate the retinol values in the children. Serum ferritin, sTfR, AGP and CRP were determined by sandwich ELISA method.[21] Urinary iodine was measured by using the Sandell-Kolthoff reaction as modified by Pino et al.[22]

Anaemia was defined as a haemoglobin concentration <13 g/dL in boys ages ≥15 years, a haemoglobin concentration <12 g/dL in children aged ≥12 years and in girls aged ≥15 years and a haemoglobin concentration <11.5 g/dL in children aged 5-11 years.[4] Iron deficiency (ID) was defined as SF<15 μg/L or sTfR concentration >7.6 mg/L.[4] Iron deficiency anaemia (IDA) was defined as simultaneous presence of ID and anaemia. Body iron stores (BIS) were estimated by the method of Cook et al.[23] Serum retinol deficiency was defined when serum retinol was less than 20 μg/dL.[24]

If the urinary iodine is less than 100 mcg/L, then the subjects are said to be iodine deficient.[25]

Validation of biochemical measurements

For the haemoglobin, serum retinol and urinary iodine estimations, the tests were measured 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 for the estimations of serum retinol was 4.7%, ferritin 2.2%; sTfR, 3.65%; CRP, 10.7%; and AGP, 4.33%, respectively.

Statistical analysis

Statistical analysis was performed with SPSS 11.0 (SPSS Inc., Chicago IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle WA, USA). Repeat measures analysis of variance with Bonferroni post hoc tests for groups with the significance set at 0.017 was used to compare the effects of the three groups x two time periods for haemoglobin, transferrin receptor, ferritin, body iron stores and serum retinol. If the interaction effect of group x time was significant ($p<0.05$), t tests between groups and paired t tests within groups were done. If there were significant $p<0.05$ differences in biochemical parameters at baseline between the groups then ANCOVA analysis was done to adjust the baseline values to a common mean and then calculate the adjusted endpoint values. When ANCOVA was done, the baseline parameters were added as covariates. Proportions were compared by using Chi-square tests. If data was not normally distributed, statistical analysis was done after log transformation, as in the case of serum ferritin. Binary logistic regression was done to compare the effects of group x time for the binary variables of anaemia, iron deficiency, iron deficiency anaemia and serum retinol deficiency. Significance was set at $p<0.05$. To analyze urinary iodine, Mann Whitney test for analysis between the different groups and Wilcoxon Signed rank test for analysis of changes within each group were used. All the retinol[26], sTfR[27], ferritin[28] and BIS[28] values were adjusted for inflammation.

RESULTS

There were 215 children in the micronutrient group, 214 children in the education group and 217 children in the control group, who completed the study. A total of 46 children in the micronutrient group, 38 children in the education group and 34 children in the control group were absent for the phlebotomy after the study was completed, although they were present for baseline phlebotomy and hence these children were excluded from the trial.

The mean age (SD) of children in the three arms at baseline was 10.1±3.02 years in the micronutrient group, 9.89±2.89 years in the education group and 9.83±3.05 years in the control group and there was no significant difference in age between the groups.

Efficacy: iron

The only significant difference in haemoglobin among the groups at baseline was the higher value in the education compared with the micronutrient group. This was the result despite the randomization. Therefore, ANCOVA analysis was done with respect to the haemoglobin analysis in the micronutrient group and education group to find out the predicted haemoglobin post intervention in these two groups if they had started at a common average haemoglobin level at baseline. If the micronutrient group and

education group had started at a baseline haemoglobin of 11.8 g/dL, then ANCOVA analysis shows that after intervention in the micronutrient group, the haemoglobin would have increased to 12.2 g/dL which is statistically significant ($p<0.05$), and in the education group, the haemoglobin would have decreased to 11.7 g/dL after intervention, though this decrease is non-significant. For haemoglobin, in ANOVA repeat measures, there was no significant difference between the three groups. This data is shown in Table 1.

Over the study period, there was a decrease in the prevalence of anaemia, and iron deficiency, only in the micronutrient group, whereas it increased in the other groups. By binary logistic regression, the changes in anaemia and iron deficiency were significantly higher in the micronutrient group compared with the control group, whereas this was not so in the education group. These data are given in Table 2.

In relation to iron deficiency anaemia, there was a decrease in the prevalence only in the micronutrient group and an increase in the other groups. By binary logistic regression we found that the micronutrient group fared significantly better than the education group. However, when the micronutrient and education groups were compared with the control group, there were no significant changes. These data are given in Table 2

In relation to ferritin, transferrin receptor and body iron stores, by ANOVA repeat measures, only the micronutrient group showed significant improvement when compared with the control, whereas the education group did not. The micronutrient group fared better than the education group too. These data are shown in Table 1.

Efficacy: vitamin A

In relation to serum retinol, by ANOVA repeat measures, only the micronutrient group showed significant improvement compared with the control, whereas the education group did not. The micronutrient group also fared better than the education group.

By binary logistic regression, there was a significant reduction of the prevalence of retinol deficiency in the micronutrient group compared with the control group, this was not so in the education group. The micronutrient group also fared better than the education group. These data are shown in Table 2.

Efficacy: iodine

It was found in this study that none of the children in the three groups had urinary iodine (UI) less than 100 mcg/L at baseline or after 8 months, indicating that none of the children were iodine deficient at baseline or post intervention. Wilcoxon signed ranks test showed that in the micronutrient group, there was no statistical significance between the baseline and post intervention UI values, but there was a significant decline in the UI values in the education group and the control group. When Mann Whitney tests were done to compare the changes between the arms, it was seen that the micronutrient group had fared significantly better than the control group and the education group, but there was no significant difference between the education group and the control group. These observations reflect the changes in non iodine deficient children (Table 1).

CRP and AGP

ANOVA repeat measures showed there was no significance in the changes in CRP in the three groups. In AGP, there were significant differences only between the education and the control groups. Overall, there was an increase in CRP in all the groups except the control, and an increase in AGP in all the groups, over the 8 months intervention period (Table 1).

DISCUSSION

The haemoglobin increase was seen only in the micronutrient group, whereas there was a decrease in the other two groups. In this study, we observed a decrease in haemoglobin and body iron stores in the control group. Similar decreases in haemoglobin in control groups of children have been seen in our earlier studies[13,15] and other studies.[29] Similar to increase in iron deficiency in the control group in this study, other studies[30] have reported increase in iron deficiency in the control group. The decrease in iron in the control group might have been due to the reduced availability of iron from food during the growing age when iron requirement is needed most.

It is also seen that by binary logistic regression, there was a reduction in the prevalence of anaemia, iron deficiency anaemia and iron deficiency only in the micronutrient group and not in the education group or in the control group. In fact in the education group and in the control group, the prevalence of anaemia, iron deficiency and iron deficiency anaemia increased post intervention compared with baseline. When transferrin receptor was considered, there was a significant reduction in transferrin receptor after intervention only in the micronutrient group, showing reduction in iron deficiency, whereas in the education group and in the control group, there was a significant increase in transferrin receptor showing increase in iron deficiency post intervention. In body iron stores too, there was an increase in body iron stores only in the micronutrient group. Thus it can be clearly seen from the analysis of haemoglobin, transferrin receptor, body iron stores, anaemia, iron deficiency and iron deficiency anaemia that only the micronutrient group was able to improve the iron status significantly. The impact of fortification on the iron status was higher, because it is a direct intervention involving no behavioural change, whereas nutrition education involves behaviour changes that would take a longer time. It might be possible that even better results could have been seen in the education group had the study been for a longer period. Moreover, there is a synergy of multiple micronutrients provided through the fortified salt in the micronutrient group. Vitamin A is necessary for iron absorption and vitamin B_{12} and folate are involved in erythopoiesis. It is the synergy of multiple micronutrients provided that is most likely to be responsible for the reduction of micronutrient deficiencies in the micronutrient group.

In relation to serum retinol, it can be seen that in the micronutrient group there was a significant improvement in the serum retinol status, whereas there were no significant changes in the serum retinol status in the other two groups. This may be because more time may be required

Table 1. Biochemical parameters after correction for inflammation in the three arms over 8 months

	Arm 1: Micronutrient group-fortified salt				Arm 2: Education group-nutrition education				Arm 3: Control group-no intervention			
	N	Baseline	Post intervention	Change (post intervention minus baseline)	N	Baseline	Post intervention	Change (post intervention minus baseline)	N	Baseline	Post intervention	Change (post intervention minus baseline)
Haemoglobin (g/dL)†	215	11.6±1.86	12.1±1.38	0.52±1.99	214	12.0±1.51	11.8±1.55	-0.22±1.75	217	11.9±1.60	11.4±2.01	-0.53±1.81
Serum retinol (µg/dL)†	154	20.2±5.07	28.8±13.9	8.56±14.6	157	20.0±5.42	21.5±10.0	1.51±9.15	140	20.9±6.74	22.6±8.77	1.65±9.98
Urinary iodine (mg/L)‡	46	400 (100-550)	335 (100-500)	-65 (-400-300)	51	400 (100-600)	200 (100-400)	-175 (-475-265)	54	400 (110-500)	200 (100-400)	-130 (-385-100)
Transferrin receptor (mg/L)†	154	9.25±3.26	8.21±3.00	-1.04±3.85	157	9.13±3.67	10.3±3.43	1.14±5.06	140	8.73±3.10	10.6±3.39	1.83±4.65
Ferritin (µg/L)†	154	x39.4±18.9	x49.9±21.5	10.8±22.7	157	x32.2±19.2	x42.2±19.9	9.49±14.9	140	x35.4±23.4	x37.5±19.0	1.09±19.2
Body iron stores (BIS) mg/kg body weight†	154	3.97±2.65	5.24±2.51	1.27±2.80	157	3.31±3.04	3.80±2.64	0.49±4.02	140	3.77±2.96	3.26±2.64	-0.51±4.05
CRP (mg/L)†	154	0.60±0.99	1.10±1.76	0.51±1.86	157	0.58±1.05	1.17±1.86	0.59±2.12	140	1.22±1.94	1.08±1.65	-0.14±1.98
AGP (g/L)†	154	0.86±0.19	1.17±0.34	0.30±0.38	157	0.84±0.21	1.15±0.28	0.31±0.30	140	0.98±0.21	1.13±0.25	0.16±0.31

†Anova repeat measures, Data given as Mean±SD; ‡median values (range), Wilcoxon signed ranks test and Mann Whitney test.
Mann Whitney test: arm 1 and arm 2, baseline p=0.799, post-intervention p=0.0001; arm 1 and arm 3 baseline p=0.880, post-intervention p=0.0001; arm 2 and arm 3, baseline p=0.502, post-intervention p=0.307.
Wilcoxon signed ranks test: arm 1 p=0.549; arm 2 p=0.0001; arm 3 p=0.0001; x Geometric mean±SD.
ANOVA repeat measures. Post hoc test for arms: Bonferroni: significance p value set at 0.017.
Haemoglobin: arm 1 and arm 2, p=1.000; arm 1 and arm 3, p=0.376; arm 2 and arm 3, p=0.255.
Serum retinol: arm 1 and arm 2, p=0.0001; arm 1 and arm 3, p=0.006; arm 2 and arm 3, p=0.827.
Serum ferritin: arm 1 and arm 2, p=0.0001; arm 1 and arm 3, p=0.0001; arm 2 and arm 3, p=1.000.
Body iron stores: arm 1 and arm 2, p=0.0001; arm 1 and arm 3, p=0.0001; arm 2 and arm 3, p=1.000.
Serum transferrin receptor (sTfR): arm 1 and arm 2, p=0.001; arm 1 and arm 3, p=0.004; arm 2 and arm 3, p=1.000.
CRP: arm 1 and arm 2, p=1.000; arm 1 and arm 3, p=0.145; arm 2 and arm 3, p=0.209.
AGP: arm 1 and arm 2, p=1.000; arm 1 and arm 3, p=0.218; arm 2 and arm 3, p=0.025.

Table 2. Prevalence percentage of serum retinol deficiency, anaemia, iron deficiency anaemia and iron deficiency in the three arms at baseline and post-intervention after correction for inflammation

	Arm 1: Micronutrient group-fortified salt				Arm 2: Education group-nutrition education				Arm 3: Control group-no intervention		
	Sample size	Baseline	Post intervention	*p* value Binary logistic regression Group x time interaction (Arm 1 with arm 3)	Sample size	Baseline	Post intervention	*p* value Binary logistic regression Group x time interaction (Arm 2 with arm 3)	Sample size	Baseline	Post intervention
Serum retinol deficiency prevalence (%)	N=154	51.6	28.1	0.0192†	N=157	56.8	51.5	0.781‡	N=140	49.0	39.0
Anaemia prevalence (%)	N=215	46.0	32.6	0.0109†	N=214	36.4	42.1	0.783‡	N=217	42.9	46.5
Iron deficiency prevalence (%)	N=154	66.9	51.3	0.0001†	N=157	56.1	79.6	0.653‡	N=140	58.6	88.6
Iron deficiency anaemia prevalence (%)	N=154	35.2	31.0	0.0913‡	N=157	22.9	36.3	0.482‡	N=140	30.0	39.3

Nutritional status: †: Significant improvement; ‡: Non-significant.
Reduction in the prevalence of vitamin A deficiency by Binary logistic regression, group x time interaction, arm 1 and arm 2, p=0.0151, arm 1 significantly more than arm 2.
Reduction in the prevalence of anaemia by Binary logistic regression, group x time interaction, arm 1 and arm 2, p=0.0042, arm 1 significantly more than arm 2.
Reduction in the prevalence of iron deficiency anaemia by Binary logistic regression, group x time interaction, arm 1 and arm 2, p=0.0175, arm 1 significantly more than arm 2.
Reduction in the prevalence of iron deficiency by Binary logistic regression, group x time interaction, arm 1 and arm 2, p=0.0001, arm 1 significantly more than arm 2.

to improve the serum retinol status through behaviour changes brought by nutrition education and the eight months of intervention might not have been sufficient.

There was no urinary iodine deficiency in the children in all the three groups at baseline or post intervention, and this study therefore shows the fluctuations in the urinary iodine values in normal non-iodine deficient children. The Government of India has made it mandatory that salt should be fortified at a level of 30 ppm of iodine at the manufacturers end. This means that 10 g of salt (which is the average consumption of salt per person per day) contains 300 µg of iodine. The RDA of iodine is 150 µg. This may be the reason for the median urinary iodine values being 200 to 400 mcg/L in this study. We added 40 ppm of iodine to the fortified salt to ensure the stability of the iodine for a period of one year, a period slightly more than the study intervention period, taking into consideration, the time for transport of the salt from Chennai, the manufacturing area to the delivery in the households in the villages.

The increase in AGP at the end of the study in all the three groups could probably be due to the onset of the rainy season with the concomitant increase in infections.

Deworming along with fortification is most likely to have been responsible for the reduction of anaemia in the children. Other studies have shown that deworming[16,17] could improve the haemoglobin status. As worms compete for the nutrients, the presence of worms itself could cause anaemia. We have eliminated this potential confounder by ensuring deworming in all the three groups. The reason why deworming has not improved the anaemic status in the control group where there was no intervention remains unclear. Since we did not do the stool analysis of the children at baseline and the end of the study, we could not estimate the worm load and this could be a limitation of this study.

We also have not done dietary assessment to assess the actual level of intake of micronutrients from the food for all the subjects in all the groups before the start of the study or during the study to find about the seasonal variations in the diets of these people. We also did not carry out the knowledge-aptitude-practice test in the subjects of the nutritional intervention group to assess the behavioural change due to the nutritional education intervention. These are all the limitations of this study.

The association between infection, inflammation and its effect on nutrient absorption, the flux of nutrients in the human body when the nutrients are provided are areas of gaps in the knowledge and further research is required in these areas.

The iron that was used in the fortification of salt was chelated ferrous sulphate. Chelated iron has a higher bioavailability than other forms of iron and this might have been one of the reasons for the improvement of the iron status in this study. Vitamin A was microencapsulated to reduce the drop in its potency and enhance its stability and shelf life and this might have contributed to the improvement in serum retinol status in the micronutrient group. The method of microencapsulation of vitamin A, vitamin B_{12} and folic acid was the same as carried out in our earlier studies[19] and the stability of vitamin A and all the other micronutrients during storage[14] and during cooking and storage[13,19] have been published earlier.

The bioavailability of all the vitamins and minerals has been studied extensively in the past when they have been delivered as supplements in the form of tablets or syrups, but what is different in this study is that these fortificants have to be stable at the high temperatures of cooking and during storage in the harsh environment of salt. We have found that all the fortificants except vitamin A are very stable during storage and cooking. Even with respect to vitamin A, its stability is considerably enhanced by microencapsulation, as in this study. With appropriate overages as was added in this study, we find that the vitamin A is highly stable and bioavailable and it has been able to bring down the prevalence of retinol deficiency significantly in the micronutrient group.

The cost of the multiple micronutrient fortified salt was just 25 paise (0.25 INR or 0.004 USD) per person per day. We feel this is a most economical way to deliver multiple micronutrients to populations.

Several reasons contributed to the absorption of the micronutrients in the micronutrient group of this study. The salt was used in cooking in all the meals. The children consumed three meals and an evening snack and therefore the micronutrients were delivered in small doses throughout the day. Malaria is not a problem in this area and hookworms too, are not normally present in this region. Hookworms thrive in cool moist soil where as the temperatures in Kariapatty is hot for most parts of the year. Ascaris infection may be commonly present and we have tackled that through periodic deworming.

Global control of multiple micronutrient deficiencies needs an integrated approach of food fortification, targeted supplementation and dietary diversification and intensive nutrition education. A stable and efficacious multiple micronutrient fortified salt could be useful in combating multiple micronutrient deficiencies in many developing countries globally. The multiple micronutrient fortified salt will be especially useful because salt is a commodity consumed universally and in about the same amounts every day. The multiple micronutrients in the salt were stable during storage and had good bioavailability.

Conclusion

It can be seen from this study that the multiple micronutrient fortified salt has been able to reduce anaemia, iron deficiency and serum retinol deficiency and improve the iron status and retinol status in this population. In this short period of eight months, nutrition education did not have a major impact in reducing anaemia or retinol deficiency.

ACKNOWLEDGEMENTS

This study was funded by Child Health Foundation, Sight and Life and Sundar Serendipity Foundation.

AUTHOR DISCLOSURES

The authors have declared no conflict of interest.

REFERENCES

1. National fact sheet, India, national family health survey(nfhs-3), ministry of health and family welfare. New Delhi:Government of India; 2006.
2. World Health Organization. Malnutrition. The global picture.

Switzerland: WHO; 2000.
3. United Nations Children's Fund. The state of the world's children 2000. New York: UNICEF; 2000.
4. WHO/UNICEF/UNU. IDA. Prevention, assessment and control. Report of a joint WHO/UNICEF/UNU consultation. Switzerland: WHO; 1998.
5. Osendarp SJ, Baghurst KI, Bryan J, Calvaresi E, Hughes D, Hussaini M et al. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. Am J Clin Nutr. 2007;86:1082-93.
6. Solon FS, Sarol JN Jr, Bernardo AB, Solon JA, Mehansho H, Sanchez-Fermin LE, Wambangco LS, Juhlin KD. Effect of a multiple micronutrient fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of school children in the Philippines. Food Nutr Bull. 2003;24:S129-40.
7. Ash DM, Tatala SR, Frongillo EA Jr, Ndossi GD, Latham MC. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. Am J Clin Nutr. 2003;77:891-8.
8. Naga TT, Winichagoon P, Dijkhuizen MA, Khan NC, Wasantwisut E, Furr H, Wieringa FT. Multi-micronutrient-fortified biscuits decreased prevalence of anaemia and improved micronutrient status and effectiveness of deworming in rural Vietnamese school children. J Nutr. 2009;139:1013-21. doi: 10.3945/jn.108.099754.
9. Muthayya S, Eilander A, Transler C, Thomas T, van der Knaap HC, Srinivasan K, van Klinken BJ, Osendarp SJ, Kurpad AV. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr. 2009;89:1766-75. doi: 10.3945/ajcn.2008.26993.
10. Osei AK, Rosenberg IH, Houser RF, Bulusu S, Mathews M, Hamer DH. Community-level micronutrient fortification of school lunch meals improved vitamin A, folate, and iron status of schoolchildren in Himalayan villages of India. J Nutr. 2010;140:1146-54. doi: 10.3945/jn.109.114751.
11. Vinod Kumar M, Rajagopalan S. Efficacy of fortification of school meals with ferrous glycine phosphate and riboflavin against anaemia and angular stomatitis in school children. Food Nutr Bull. 2009;30:260-4.
12. Vinod Kumar M, Rajagopalan S. Impact of a multiple-micronutrient food supplement on the nutritional status of school children. Food Nutr Bull. 2006;27:203-10.
13. Vinodkumar M and Rajagopalan S. Multiple micronutrient fortification of salt. Eur J Clin Nutr. 2009;63:437-45. doi: 10.1038/sj.ejcn.1602955.
14. Vinodkumar M, Erhardt JG, Rajagopalan S. Impact of a multiple-micronutrient fortified salt on the nutritional status and memory of school children. Int J Vitam Nutr Res. 2009; 79:348-61. doi: 10.1024/0300-9831.79.56.348.
15. Kumar MV, Rajagopalan S. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. Asia Pac J Clin Nutr. 2007;16:505-11.
16. Olsen A, Thiong'o FW, Ouma JH, Mwaniki D, Magnussen P, Michaelsen KF, Friis H, Geissler PW. Effects of multi micronutrient supplementation on helminth reinfection: a randomized, controlled trial in Kenyan schoolchildren. Trans R Soc Trop Med Hyg. 2003;97:109-14. doi: 10.1016/S0035-9203(03)90042-3.
17. Taylor M, Jinabhai CC, Couper I, Kleinschmidt I, Jogessar VB. The effect of different antihelminthic treatment regimens combined with iron supplementation on the nutritional status of school children in KwaZulu-Natal, South Africa: a randomized controlled trial. Trans R Soc Trop Med Hyg. 2001;95:211-6. doi: 10.1016/S0035-9203 (01) 90171-3.
18. Gopalan C, Rama Sastri BV, Balasubramanian SC. Nutritive value of Indian foods. Indian Council of Medical Research. Hyderabad: National Institute of Nutrition Press; 1999.
19. Vinod Kumar M, Rajagopalan S. Trial using multiple micronutrient food supplement and its effect on cognition. Indian J Pediatr. 2008;75:671-8. doi: 10.1007/s12098-008-0127-1.
20. Dacie JV, Lewis SM. Practical Haematology. Edinburgh: Churchill Livingstone; 1995.
21. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and Creactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr. 2004;134:3127-32.
22. Pino S, Fang SL and Braverman LE. Ammonium persulphate: a safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem. 1996;42:239-43.
23. Cook JD, Flowers CH and Skikne BS. The quantitative assessment of body iron. Blood. 2003;101:3359-64. doi: 10.1182/blood-2002-10-3071.
24. Alfred Sommer, Frances R Davidson. Assessment and control of vitamin A deficiency: the annecy accords. J Nutr. 2002;132:2845S-50S
25. WHO, UNICEF, ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination, A guide for programme managers. Switzerland: WHO; 2007.
26. Thurnham DI, McCabe GP, Northrop-Clewes CA, Neste lP. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet. 2003;362:2052-8. doi: 10.1016/S0140-6736(03)15099-4.
27. Thurnham DI, Mburu AS, Mwaniki DL, Muniu EM, Alumasa F, de Wagt A. Using plasma acutephase protein concentrations to interpret nutritional biomarkers in apparent-ly healthy HIV-1 seropositive Kenyan adults. Br J Nutr. 2008;100:174-82. doi: 10.1017/S0007114507883012.
28. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010;92:546-55. doi: 10.3945/ajcn.2010.29284.
29. Nair KM, Brahmam GNV, Ranganathan S, Vijayaraghavan K, Sivakumar B, Krishnaswamy K. Impact evaluation of iron and iodine fortified salt. Indian J Med Res. 1998;108: 203-11.
30. Zimmermann MB, Wegmueller R, Zeder C, Chaouki N, Rohner F, Saissi M, Torresani T, Hurrell RF. Dual fortification of salt with iodine and micronised ferric pyrophosphate, a randomized double blind controlled trial. Am J Clin Nutr. 2004;80:952-9.

Original Article

An efficacy study on alleviating micronutrient deficiencies through a multiple micronutrient fortified salt in children in South India

Malavika Vinod Kumar PhD[1], Praveen K Nirmalan MPH[2], Juergen G Erhardt PhD[3], Lakshmi Rahmathullah MBBS, DTPH[4], S Rajagopalan PhD[5]

通过复合微量营养素强化盐来缓解印度南部儿童微量营养素缺乏的效果研究

背景：在印度普遍存在多重微量营养素缺乏。目的：本研究的目的是与营养教育干预和无干预相比，确定复合微量营养素强化盐在解决泰米尔纳德邦儿童多重微量营养素缺乏的效果。方法：本研究采用社区为基础的为期 8 个月的随机对照试验设计，研究复合微量营养素盐（微量营养素组）、营养教育（教育组）和无干预（对照组），对血红蛋白、血清铁蛋白、可溶性转铁蛋白受体、体内铁储存和维生素 A 和尿碘的影响。强化盐含有铁、碘、维生素 A、维生素 B_{12} 和叶酸。对所有的儿童在基线和研究末期生化测量之前进行驱虫。结果：与对照组相比，微量营养素组的多数生化参数显著改善，然而这种改善在教育组和对照组中未被发现。8 个月以后，微量营养素组血红蛋白增加了 0.52 g/dL，维生素 A 增加了 8.56 μg/dL，铁蛋白增加了 10.8 μg/dL，体内铁储存增加了 1.27 mg，维生素 A 缺乏症的发生率从 51.6%下降到 28.1%，贫血的发生率从 46.0%下降到 32.6%，铁缺乏的发生率从 66.9%下降到 51.3%，缺铁性贫血的发生率从 35.2%下降到 31.0%，然而在其它两组未见显著的缺乏增加或改变。结论：复合微量营养素强化盐能够改善铁和维生素 A 的状态，然而在营养教育组未见这种改善。

关键词：复合微量营养素强化盐、微量营养素缺乏、生化评估、儿童、印度