

Improving Micronutrient Status of Children and Women in Rural Communities in India Using Crystal Salt Enriched with Multiple Micronutrients

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Summary To demonstrate that fortified crystal salt enriched with iron, iodine, vitamin B12, folic acid and zinc can combat multi-micronutrient deficiencies. A randomized controlled study was conducted in 6 villages in Tiruvallur district, in Tamilnadu, South India. All the women and children aged 5–17 y in households in the experimental villages ($n=117$) were provided the fortified salt for 8 mo. Similar demographic group in the control villages ($n=95$) used regular non-fortified salts for the same time period. Blood from study subjects were analysed for hemoglobin, serum ferritin, serum transferrin receptor, AGP, CRP, and serum zinc, at the beginning and end of the study. Urine was analyzed for iodine at the same times. The experimental group showed a statistically significant increase in hemoglobin (>1.05 g/dL), serum zinc (>12.23 μ g/dL), ferritin (>6.97 μ g/L) and body iron stores (>0.73 mg/kg body weight), compared to the control group. A significant decrease in the prevalence of anaemia from 67.5% to 29.1% and zinc deficiency from 32.7% to 12.4% was observed in the experimental group relative to control group, using Binary logistic regression. There was no change in urinary iodine in the experimental group while it decreased significantly in the control. The fortified crystal salt was effective in decreasing multi-micronutrient deficiencies.

Key Words multiple micronutrient fortified salt, micronutrient deficiencies, biochemical assessment, women and children in India, crystal salt fortification

Micronutrient deficiencies causing “hidden” hunger is a major public health problem in many developing countries including India. Even in developed countries like the United States of America, surveys (NHANES) from 2003 to 2012 estimated that an average of 5.6% of the population met the criteria for anemia and 1.5% for moderate-severe anemia during this 10-y period. The National family health survey done by the Government of India in 2015–2016 shows that in Tamilnadu, 55.1% of women and 20.4% of men are anaemic (1). One reason for anaemia and micronutrient deficiency is the consumption of plant based cereal diets (2, 3). Dietary phytate present in both rice and wheat, the two important cereals consumed by Indians, inhibits the absorption of many micronutrients, notably iron and zinc. In India, amongst the poor, there is also very little consumption of meat and dairy products, which have bioavailable haeme iron and vitamin B12, due to its high cost. 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) to combat multiple micronutrient deficiencies.

In our earlier studies, we have shown that iron and iodine can be provided through double fortified salt in

rural villages, demonstrating that salt is an ideal vehicle for delivery of micronutrients (9). Multiple micronutrients could be used to fortify salt (10–12). We have shown that the prevalence of serum retinol deficiency in children in Tamilnadu is 57.1% (11), and prevalence of angular stomatitis due to B complex deficiencies was 12.8% (11). This reconfirms that multiple micronutrient deficiencies exist in children and adults in India and needs to be combated. For salt fortification to be successful, the micronutrients should not change the color, odor, or taste of the food, should be stable at cooking temperatures, and should be bioavailable. All of these were achieved in our multiple micronutrient fortified salt (10–12). All our earlier studies were done using refined or purified powder salt, which has low moisture and very little impurities. However, the poor consume only crystal salt, which is unrefined, has a high moisture content and also presence of other compounds of calcium and magnesium. Therefore, in this study, we developed methods for fortifying crystal salt with multiple micronutrients and tested its efficacy and bioavailability in a cohort, where micronutrient deficiency was observed.

Aim. To combat a major public health problem of hidden hunger caused due to multiple micronutrient deficiencies in villages in Tiruvallur district, in Tamilnadu, South India using a multiple micronutrient forti-

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fied crystal salt enriched with iron, iodine, vitamin B12, folic acid and zinc.

MATERIALS AND METHODS

Study location. The study was done in 6 villages in Tiruvallur district in Tamilnadu, South India.

Study design. The study was a randomized controlled trial to study the efficacy of the multiple micronutrient fortified crystal salt in combating multiple micronutrient deficiencies.

Randomisation. The villages were chosen from a list of villages in the Tiruvallur district. The villages were chosen from a computer generated random table and were assigned to the experimental or control group. After randomization, we verified whether the families in the villages had similar socioeconomic status.

Inclusion criteria. This study included children aged 5 to 17 y and all the women in the households after obtaining written informed consent from the head of the household.

Exclusion criteria. This study excluded subjects where the head of the household did not provide informed consent. Subjects who had a hemoglobin level less than 8 g/dL (defined as severe anemia) 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 procedure involving human subjects were approved by the Ethics committee of Sundar Serendipity Foundation (number 15012012). Written informed consent was obtained from all the subjects who participated in the study.

Deworming. All members of all the households enrolled in the study in both the experimental and control groups were given a tablet of albendazole (400 mg) at baseline and post intervention after 8 mo. 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 as in other studies (13, 14).

Experimental and control groups. The households in the experimental villages received the multiple micronutrient fortified salt from April 2012 to beginning of January 2013, a period of 8 mo. The households in the control villages continued to use the conventional iodised salt they were using before the study. Blood samples were collected in all the participants (only the women and children in the households) before the start of the study and at the end of the study. Urine samples were collected in the same subsample of the subjects at the same times blood was collected. The tests done are hemoglobin, serum ferritin, soluble transferrin receptor (sTfR), alpha glycoprotein (AGP), C reactive protein (CRP), urinary iodine and serum zinc.

To all the heads of the households and the women in the experimental households, the design of the study was explained and they were 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 mo till the study concluded. They were shown an educational film on the role of micronutrients in human health and the importance of the fortified salt in combating micronutrient deficiencies. All these households were provided with the multiple micronutrient fortified salt every month for a period of 8 mo. From our earlier studies on the fortified salt we were aware that the average salt consumption per person per day is about 10 g.

Before the start of the study, we checked for the consumption of salt and found that the consumption of salt in both the experimental and control groups was similar. The range was from 7–11 g of salt and the average was about 10 g per person per day.

From the size of the households we could calculate the average salt consumption per household 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 determined the amount of fortified salts used by the families, provided by us. It was found that all the families were using the fortified salt provided by us only.

Sample size. We have considered a *p*-level 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 hemoglobin from 0.5 to 0.7 g/dL between the experimental and control groups, with a standard deviation of about 1 (10–12). With respect to serum retinol, our earlier studies have seen a mean increase of 4.5 μ g/dL, between the experimental and control groups, with a standard deviation of 7 (11). Taking into considerations our earlier studies on multiple micronutrient fortified salt for changes in serum zinc, ferritin and sTfR, we arrived at a sample size of 90 in each arm.

Subjects recruited and those who actually completed the study. There were 117 women and children in the experimental group and 95 women and children in the control group who were present for both baseline and endline analysis after excluding those who were treated with iron tablets for ethical reasons because their hemoglobin was less than 8 g/dL and those who were not present for both baseline and endline blood tests. Eighteen subjects in the experimental group and 21 subjects in the control group were present for baseline blood tests but were absent during endline blood tests and were therefore not included in the analysis. Urinary Iodine was done in a subsample of 52 subjects in the experimental group and 65 subjects in the control group.

Dosage of micronutrients. The salt was used in all the meals prepared in the household. It was found out that the average consumption of salt was 10 g per person per day. Therefore the fortified salt was prepared such that 10 g of the fortified salt contained about 1 RDA (Recommended dietary allowance) of the micronutrients, except iron which given at a dosage of 10 mg per day (about 50% RDA) as the iron was chelated, instead

of 22 mg iron which was the RDA. Ten grams of the fortified salt contained 10 mg of chelated iron, 400 μg of iodine, 4 μg of vitamin B12 and 100 μg of vitamin folic acid and 10 mg zinc. The iron used was chelated ferrous sulphate for enhanced bioavailability and the rest of the micronutrients were microencapsulated to prevent interaction amongst them as in our previous studies (10–12).

Chelated ferrous sulphate was obtained by chelating ferrous sulphate with sodium hexa meta phosphate and malic acid. The iodine source was potassium iodate which was encapsulated in cellulose acetate phthalate. Zinc source was zinc oxide. The vitamin sources were cyanocobalamine (vitamin B12) and folic acid. Since vitamin B12 is magenta in colour, and since folic acid is turmeric yellow in color, and they would impart these colours to the fortified salt, which was not acceptable and hence, they were coated with food grade titanium dioxide to mask the colors. They were then coated with cellulose acetate phthalate and after that blended with the salt.

Blood collection and storage. Venous blood samples (5 mL) were drawn from the women and children at the central area in the villages and 500 μL transferred into vials with ethylenediaminetetraacetate (EDTA) as an anticoagulant. The hemoglobin measurements were performed on these samples within a few hours of blood collection at the central area in the villages. The remaining 4.5 mL of blood was transferred into vials and the blood was allowed to clot. Serum separation was performed in the laboratory and the samples were frozen at -20°C for further analyses. It was a random blood sample as fasting blood sample is not required for the tests.

Laboratory analyses. The biochemical estimations done were for hemoglobin, serum ferritin, sTfR, CRP, AGP, serum zinc and urinary iodine. Hemoglobin, serum zinc, serum ferritin (SF), sTfR, CRP and AGP was measured in all the subjects in both the groups, before the start of the study and at the end of the study. Data from all the subjects were used for statistical analysis after making corrections for inflammation. Urinary iodine was done in a random sub sample. Pretest and post intervention urinary iodine tests were performed on the same random subsample of subjects.

Hemoglobin was estimated by the cyanmethemoglobin method with a colorimeter (15). Serum ferritin, sTfR, AGP and CRP was determined by sandwich ELISA method (16) in our collaborator's Laboratory in Germany. The serum samples were transported on dry ice from India to Germany. Urinary iodine was measured by using the Sandell-Kolthoff reaction as modified by Pino et al. (17). Serum zinc was measured by induction coupled plasma optical emission spectrometer (ICP-OES) at an independent certified laboratory in Chennai.

Validation of biochemical measurements. In hemoglobin, serum zinc and urinary iodine, estimations were measured in duplicate in 10% of the samples. Serum ferritin, sTfR, CRP, AGP were measured in duplicate for all the samples. The coefficient of variation for estimation of ferritin was 2.06%; sTfR was 2.6%; CRP was

4.98%; and AGP was 4.42%.

Anaemia was defined as a hemoglobin concentration $<13\text{ g/dL}$ in boys aged $\geq 15\text{ y}$, a hemoglobin concentration $<12\text{ g/dL}$ in children aged $\geq 12\text{ y}$ and in girls and women aged $\geq 15\text{ y}$ and a hemoglobin concentration $<11.5\text{ g/dL}$ in children aged 5–11 y (18). Iron deficiency (ID) was defined as serum ferritin $<15\text{ }\mu\text{g/L}$ or sTfR concentration $>7.6\text{ mg/L}$ (18). Iron deficiency anemia (IDA) was defined as simultaneous presence of ID and anemia (18). Body iron stores were estimated by the method of Cook et al. (19).

If the urinary iodine is less than 100 $\mu\text{g/L}$, then the subjects are said to be iodine deficient, else they are iodine sufficient (20).

If serum zinc is less than 66 $\mu\text{g/dL}$, then the subjects are said to be serum zinc deficient, else they are serum zinc sufficient (21).

Inflammation adjustments. Inflammation increases ferritin values. The subjects were grouped at baseline and endline according to their inflammation status (22, 23) as follows: When there is no increase in the acute phase proteins ($\text{CRP} \leq 5\text{ mg/L}$ and $\text{AGP} \leq 1\text{ g/L}$) it is called the reference group and the ferritin, sTfR, and body iron stores are used as it is. When only the CRP is increased ($\text{CRP} > 5\text{ mg/L}$) and AGP is normal ($\text{AGP} \leq 1\text{ g/L}$), it is called the incubation group and all the ferritin values in this group is multiplied by the correction factor 0.77. When both the CRP and AGP are increased ($\text{CRP} > 5\text{ mg/L}$ and $\text{AGP} > 1\text{ g/L}$), this group is called the early convalescence group and all the ferritin values are multiplied by 0.53. When CRP is normal ($\text{CRP} \leq 5\text{ mg/L}$) and AGP is raised ($\text{AGP} > 1\text{ g/L}$), this group is called the late convalescence group and the ferritin values are multiplied by 0.75. For sTfR, the subjects are grouped into the above inflammation groups (reference, incubation, early convalescence and late convalescence) and the geometric mean of each group is found out. The correction factor (CF) for each group is calculated as follows: $\text{CF} = \text{Geometric mean of reference group} / \text{geometric mean of the respective inflammation group}$. The correction factor is then multiplied to the values of the respective groups. The inflammation corrected ferritin and sTfR results are used to calculate body iron stores.

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 experimental group was compared with the control group to compare the efficacy of the intervention. Thus the efficacy of the multiple micronutrient-fortified salt in combating micronutrient deficiencies was studied. Repeat measures Analysis of variance was used to compare the effects of group \times time for hemoglobin, sTfR, ferritin, body iron stores, CRP, AGP and serum zinc. If the interaction effect of group \times time was significant ($p < 0.05$), t tests between groups and paired t tests within groups were done. Proportions were compared by using Chi-square tests. If data was not normally distributed, statistical analysis was done after log transformation. Binary logistic regression was done to compare the effects of group \times time for the

Table 1. Stability of the micronutrients in the multiple micronutrient fortified salt for 1 y at 30°C and 45% relative humidity. (Mean of the 2 batches prepared for the study)

Nutrients ¹	Label claim of the micronutrients in 10 g salt	Initial levels on the date of manufacture	Levels after 6 mo from the date of manufacture	Levels after 12 mo from the date of manufacture
Iron (ppm)	1,000	1,079	1,045	1,050
Iodine (ppm)	30	42	39	37
Vitamin B12 (μg)	4	4.4	4.3	4.1
Folic acid (mg)	100	100.9	100.5	100.2
Zinc (mg)	10	10.5	10.6	10.2

¹ Iron was determined by potassium thiocyanate method and the red colour developed was measured in a colorimeter. Iodine was determined by titrating with sodium thiosulphate solution. Zinc was determined by colour developed with dithionite in chloroform which was read in a colorimeter. Folic acid was determined by colour developed with a dye (1-naphthyl ethylene diamine dihydrochloride) in iso butyl alcohol and read in a colorimeter. Vitamin B12 was measured by microbiology with the cup plate method using *Escherichia coli* NCIM 2068.

binary variables of anaemia, iron deficiency, iron deficiency anaemia and serum zinc 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.

RESULTS

Microencapsulation and stability of the fortified salt

All the multiple micronutrients except iron and zinc were microencapsulated as in our previous studies (10–12). The micronutrients were microencapsulated to prevent their interaction with each other and their interaction with the salt. These interactions could lead to the loss of their potency as in the case of iodine. The other reason for microencapsulation was to mask their color as in the case of vitamin B12 and folic acid. Vitamin B12 is magenta in color and folic acid is yellow in color and their colors have to be masked so that their colors are not imparted to the salt which is white in color. Since crystal salt is not purified or refined, it contains higher amounts of moisture and other impurities when compared to refined salt. The microencapsulation of the micronutrients ensures their stability even under these extreme conditions. The stability of the micronutrients in the fortified salt was assessed on the date of manufacture, after 6 mo and after 1 y from the date of manufacture. It was seen that all the micronutrients are stable for more than a year in the crystal salt. These data are given in Table 1.

Inflammation status

There was no inflammation in 62% of the experimental group and 60% of the control group at baseline. Post intervention, there was no inflammation in 73% of the experimental group and 74% of the control group.

Efficacy study

The experimental group showed a statistically significant increase in hemoglobin (>1.05 g/dL), serum zinc (>12.23 $\mu\text{g}/\text{dL}$), ferritin (>6.97 $\mu\text{g}/\text{L}$) and body iron stores (>0.73 mg/kg body weight), compared to the control group. A significant decrease in the prevalence of anaemia from 67.5% to 29.1% and zinc deficiency

from 32.7% to 12.4% was observed in the experimental group relative to control group, using Binary logistic regression. There was no change in urinary iodine in the experimental group while it decreased significantly in the control (Tables 2, 3).

There was a significant improvement in the experimental group when compared to the control group in hemoglobin, serum zinc, ferritin and body iron stores by anova repeat measures showing that iron and zinc were absorbed from the fortified salt. There were no significant changes in soluble transferrin receptor (sTfR), CRP and AGP in both the groups (Table 2). CRP and AGP are inflammation markers and generally no significant changes are expected between the experimental and control groups in these. We found no differences in sTfR between both the groups. The reason for this is not clear.

There was a significant decrease in the prevalence of zinc deficiency and anaemia, in the experimental group when compared with the control showing that iron and zinc were absorbed from the fortified salt. There were no significant changes in the prevalence of iron deficiency or iron deficiency anaemia (Table 3) and a period of more than 8 mo may be required to show significant changes.

Urinary iodine

At baseline, Mann Whitney test showed that there was no significant difference in the median urinary iodine (UI) in both the groups, so both the groups were homogenous. Over the study period, Wilcoxon signed ranks test showed that there was no significant change in median UI in the experimental group whereas there was a significant decrease in the median UI in the control group. At the end of the study, Mann Whitney test showed that there was a significant difference in UI between the experimental and control groups. These data are shown in Table 2. This study shows that the fortified salt was able to maintain the UI at the same levels in the experimental group whereas the control group showed significant decrease in UI.

Table 2. Biochemical changes in the experimental and control group over 8 mo.

Parameter	Experimental group: fortified salt			ANOVA repeat measures (Experimental with control)		Control group: no intervention			
	n	Baseline	Post intervention	Change (post intervention minus baseline)	p value	n	Baseline	Post intervention	Change (post intervention minus baseline)
Hemoglobin g/dL	117	11.35±1.30	12.4±1.37	1.05±1.08	0.0001 ^a	95	11.72±1.47	11.67±1.44	-0.05±0.44
Serum zinc µg/dL	117	75.44±18.42	87.67±25.83	12.23±30.30	0.040 ^a	95	84.05±25.32	86.66±28.11	2.61±36.81
Urinary iodine ¹ µg/L	52	260 (90-550)	255 (80-565)	-55 (-405-415)	0.0001 ^a	65	292 (85-465)	100 (67-360)	-115 (-425-160)
Soluble transferrin receptor mg/L	117	6.55±2.88	6.13±2.42	-0.425±2.21	0.798 ^b	95	8.23±4.3	7.73±4.12	-0.501±1.88
Ferritin ² µg/L	117	32.42±24.72	38.13±28.51	6.97±19.07	0.004 ^a	95	26.18±26.78	25.63±22.31	-2.08±13.84
Body iron stores (BIS) mg/kg body weight	117	4.57±3.37	5.30±3.06	0.73±2.10	0.031 ^a	95	3.02±4.08	3.17±3.97	0.16±1.46
CRP mg/L	117	2.500±4.09	2.800±5.37	0.30±5.48	0.158 ^b	95	2.730±5.07	1.985±2.446	-0.745±4.75
AGP g/L	117	0.932±0.300	0.868±0.268	-0.064±0.321	0.548 ^b	95	0.956±0.294	0.866±0.216	-0.09±0.282

All data given as mean ±SD unless otherwise indicated.

¹Median (range). Wilcoxon signed ranks test and Mann Whitney test.

Mann Whitney test: experimental and control, baseline $p=0.516$, endline $p=0.0001$.

Wilcoxon signed ranks test: experimental $p=0.122$, control $p=0.0001$.

²Geometric mean ±SD.

^a Significant improvement. ^b Non significant.

Table 3. Prevalence percentage of serum zinc deficiency, anaemia, iron deficiency anaemia and iron deficiency in the experimental and control groups at baseline and post intervention after correction for inflammation.

	Experimental group: fortified salt			Control group: no intervention			p value	
	Sample size	Baseline	Post intervention	Sample size	Baseline	Post intervention		Binary logistic regression Group×time interaction (Experimental group with control group)
Serum zinc deficiency prevalence %	n=117	32.7	12.4	n=95	22.1	18.9	0.018 ^a	
Anaemia prevalence %	n=117	67.5	29.1	n=95	46.3	51.6	0.0001 ^a	
Iron deficiency prevalence %	n=117	26.8	23.2	n=95	43.2	38.6	1.000 ^b	
Iron deficiency anaemia prevalence %	n=117	20.5	9.8	n=95	26.1	26	0.135 ^b	

Nutritional status. ^a Significant improvement. ^b Non significant.

DISCUSSION

We have shown in earlier studies that multiple micronutrient fortification of refined powder salt is feasible and a practical solution to fight hidden hunger (10–12). However the poor consume only unrefined crystal salt which has high moisture content. Nobody has fortified crystal salt with multiple micronutrients before. In this study we have fortified crystal salt with five micronutrients (iron, iodine, B12, folic acid and zinc) and we have shown that all the micronutrients in the fortified salt are stable for a year (Table 1) and that three micronutrients (iron, iodine and zinc) are bioavailable and their levels have improved significantly in the experimental subjects when compared to the control (Tables 2, 3).

In this study, though the fortified salt caused a significant decline in the prevalence of anaemia and zinc deficiency in the experimental group, there was no significant change in the prevalence of iron deficiency or iron deficiency anaemia (Table 3). The reason for this is not clear and it may be that the study period of 8 mo might have been insufficient to decrease the prevalence of iron deficiency and iron deficiency anaemia, and a longer study period may have proven to be beneficial.

Unlike other fortification programmes, salt fortification has enormous benefits because salt is used by every family, everyday during cooking, and the entire family gets the daily requirement of these micronutrients. No other fortification targets the entire family like salt fortification. In India, where 50% of women, 70% of young children and 25% of men are anaemic (1), salt fortification with multiple micronutrients could be one of the ways to tackle hidden hunger caused due to multiple micronutrient deficiencies.

We had to fortify iodine at 40 ppm level because the Government of India mandates that the iodine in fortified salt should be at least 30 ppm at the manufacturing area. We have added 40 ppm iodine to ensure stability of iodine in the fortified salt for a period of 1 y, as in our earlier studies on fortified salt (10–12). We did not test the iodine content in the salt used by the control group households. If we had done that we might have known as to why there was a decrease in urinary iodine in the control group. This is one of the drawbacks of this study.

There is a synergy among the multiple micronutrients provided by the fortified salt. It is not just the iron in the fortified salt that combats anaemia, but vitamin B12 and folic acid too are necessary for erythropoiesis. Therefore, it may be possible that the iron, vitamin B12 and folic acid in the fortified salt helped combat anaemia in the experimental group. Deworming along with fortification has been responsible for the reduction of anaemia in the experimental group. As worms compete for the nutrients, the presence of worms itself could cause anaemia. We have eliminated this potential confounder by ensuring deworming in both the experimental and control groups. The iron that was used in the fortification of salt was chelated ferrous sulphate as in

our earlier studies (9–12). Chelated iron has a higher bioavailability than ordinary iron and this might have been one of the reasons for the improvement of the iron status in this study (24).

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 find that all the 5 micronutrients were stable during storage in the fortified salt.

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 the most economical way to deliver multiple micronutrients to populations.

Several reasons contributed to the absorption of the micronutrients in the experimental group. The salt was used in cooking in all the meals. The women and children consumed three meals and therefore the micronutrients were delivered in small doses throughout the day. The multiple micronutrient fortified salt will be especially useful because salt is a commodity consumed universally and in about the same amounts every day.

There are some limitations in this study. Though the salt is fortified with iron, iodine, vitamin B12, folic acid and zinc, we did not assess vitamin B12 and folic acid in women and children due to lack of funds. Hence future studies assessing these vitamins are necessary. This was also not a double blind randomized control study, which is a gold standard. We had to resort to this randomized control study, where we did no intervention in the control group. This was once again due to the limited funding we had to do this study.

Public health implications

Hidden hunger due to micronutrient deficiencies is a huge public health problem in developing countries. Anaemia affects nearly 50% of the women and young children in India. The current methods to tackle this problem by the government are to give iron tablets to pregnant women and children. Though entire families suffer from the problem of hidden hunger, only specific targets like pregnant women and school children are targeted, resulting in persistence of the problem for several decades. Our solution is unique and it provides multiple micronutrients through the medium of salt, which is used by the entire population every day. Thus, the micronutrients are made available to the entire families, communities and entire populations, and so reduction in anaemia is seen in few months. Thus, this paper helps to show a path to resolve the huge public health problem of overcoming hidden hunger due to multiple micronutrient deficiencies.

CONCLUSION

The multiple micronutrient fortified crystal salt has been efficacious reducing the prevalence of anaemia and zinc deficiency and improving hemoglobin, serum ferritin, body iron stores and serum zinc and maintaining urinary iodine at the same levels in the population

which consumed the fortified salt.

Authorship

Research conception and design: MV; experiments: MV, JE; statistical analysis of the data: MV; interpretation of the data: MV; writing of the manuscript: MV.

Child Health Foundation and Harvest Plus had no role in the design, analysis or writing of this article.

Field work was done under Sundar Serendipity Foundation and Laboratory work under The Micronutrient Research Foundation in India and VitMin lab in Germany.

Disclosure of state of COI

There are no conflicts of interests.

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REFERENCES:

- 1) Government of India. 2016. National fact sheet, India, National family health survey (NFHS-4). Ministry of Health and Family Welfare, New Delhi.
- 2) World Health Organization. 2000. Malnutrition. The Global Picture. WHO, Geneva.
- 3) United Nations Children's Fund. 2000. The state of the world's children 2000. UNICEF, New York.
- 4) WHO/UNICEF/UNU/IDA. 1998 Prevention, assessment and control. Report of a joint WHO/UNICEF/UNU consultation. WHO, Geneva.
- 5) Osendarp SJM, Baghurst KI, Bryan J, Calvaresi E, Hughes D, Hussaini M, Karyadi SJM, van Klinken BJ-W, van der Knaap HCM, Lukito W, Mikarsa W, Transler C, Wilson C, NEMO Study Group. 2007. Effect of a 12-month 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* **86**: 1082–1093.
- 6) Solon FS, Sarol JN Jr, Bernardo AB, Solon JA, Mehansho H, Sanchez-Fermin LE, Wambangco LS, Juhlin KD. 2003. Effect of a multiple micronutrient fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. *Food Nutr Bull* **24**: S129–S140.
- 7) Ash DM, Tatala SR, Frongillo EA Jr, Ndossi GD, Latham MC. 2003. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. *Am J Clin Nutr* **77**: 891–898.
- 8) Nga TT, Winichagoon P, Dijkhuizen MA, Khan NC, Wasantwisut E, Furr H, Wieringa FT. 2009. Multi-micronutrient-fortified biscuits decreased prevalence of anemia and improved micronutrient status and effectiveness of deworming in rural Vietnamese school children. *J Nutr* **139**: 1013–1021.
- 9) Vinodkumar M, Rajagopalan S, Bhagwat IP, Singh S, Parmar BS, Mishra OP, Upadhyay SS, Bhalia NB, Deshpande SR. 2007. A multicenter community study on the efficacy of double-fortified salt. *Food Nutr Bull* **28**(1): 100–108.
- 10) Vinodkumar M, Rajagopalan S. 2009. Multiple micronutrient fortification of salt. *Eur J Clin Nutr* **63**: 437–445.
- 11) Vinodkumar M, Erhardt JG, Rajagopalan S. 2009. Impact of a multiple-micronutrient fortified salt on the nutritional status and memory of schoolchildren. *Int J Vitam Nutr Res* **79**: 348–361.
- 12) Kumar MV, Rajagopalan S. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia Pac J Clin Nutr* **16**: 505–511.
- 13) Olsen A, Thiong'o FW, Ouma JH, Mwaniki D, Magnusen P, Michaelsen KF, Friis H, Geissler PW. 2003. Effects of multimicronutrient supplementation on helminth reinfection: a randomized, controlled trial in Kenyan schoolchildren. *Trans R Soc Trop Med Hyg* **97**: 109–114.
- 14) Taylor M, Jinabhai CC, Couper I, Kleinschmidt I, Jogeswar VB. 2001. The effect of different anti helminthic treatment regimens combined with iron supplementation on the nutritional status of schoolchildren in KwaZulu-Natal, South Africa: a randomized controlled trial. *Trans R Soc Trop Med Hyg* **95**: 211–216.
- 15) Dacie JV, Lewis SM. 1995. Practical Haematology. Churchill Livingstone, Edinburgh.
- 16) Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. 2004. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immune sorbent assay technique. *J Nutr* **134**: 3127–3132.
- 17) Pino S, Fang SL, Braverman LE. 1996. Ammonium persulphate: a safe alternative oxidizing reagent for measuring urinary iodine. *Clin Chem* **42**: 239–243.
- 18) UNICEF/WHO/UNU/MI. 1998. Preventing iron deficiency in women and children: Background and consensus on key technical issues and resources for advocacy, planning and implementing national programmes. p. 10. International Nutrition Foundation, Boston, MA.
- 19) Cook JD, Flowers CH, Skikne BS. 2003. The quantitative assessment of body iron. *Blood* **101**: 3359–3364.
- 20) World Health Organization, United Nations Children's Fund & International Council for the Control of Iodine Deficiency Disorders. 2007. Assessment of iodine deficiency disorders and monitoring their elimination 3rd ed. WHO, Geneva.
- 21) Hess SY, Peerson JM, King JC, Brown KH. 2007. Use of serum zinc concentration as an indicator of population zinc status. *Food Nutr Bull* **28**(3 Suppl): S403–S429.
- 22) Thurnham DI, Mburu AS, Mwaniki DL, Muniu EM, Alumasa F, de Wagt A. 2008. Using plasma acute-phase protein concentrations to interpret nutritional biomarkers in apparently healthy HIV-1-seropositive Kenyan adults. *Br J Nutr* **100**: 174–182.
- 23) Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. 2010. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr* **92**: 546–555.
- 24) Milman N, Jønsson L, Dyre P, Pedersen PL, Larsen LG. 2014. Ferrous bisglycinate 25 mg iron is as effective as ferrous sulfate 50 mg iron in the prophylaxis of iron deficiency and anemia during pregnancy in a randomized trial. *J Perinat Med* **42**(2): 197–206.