

Original Article

Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children

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Aim: To test the efficacy of a multiple micronutrient fortified salt in improving the micronutrient status and health of school children and its effect on cognition.

Methods: A salt fortified with multiple micronutrients was developed containing chelated ferrous sulphate, microencapsulated vitamin A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate and iodine. The efficacy of the fortified salt was assessed in 7-11 year old school children in Chennai, India. In the experimental group (N=63), the food in the school kitchen was cooked with the fortified salt for a period of one year. The control group (N=66) consisted of day scholars who did not eat at the school. Hemoglobin, red blood cell count, hematocrit, serum vitamin A, urinary iodine and prevalence of angular stomatitis were measured at baseline and at the end of the study after one year. A battery of 7 memory tests (The personal information test, the Mann-Suiter Visual memory screen for objects, The digit span forward test, The digit span backward test, The delayed response test, The Benton Visual Retention Test and The Cattells retentivity test), one test for attention and concentration (Letter cancellation test) and one test for intelligence (Raven's coloured progressive matrices) were administered to all the children at baseline and endline.

Results: There was a significant ($p < 0.05$) improvement in the experimental group in hemoglobin, red cell count, urinary iodine and serum vitamin A whereas in the control group there was a statistically significant decline ($p < 0.05$) in hemoglobin, hematocrit, red cell count and urinary iodine. Angular stomatitis was eliminated from baseline 30.4% in the experimental group whereas it increased from 3.25% to 25.5% in the control group. In 4 tests out of the 7 memory tests and in the letter cancellation test for attention, the mean increment in scores in the experimental group is significantly more ($p < 0.05$) than the control group. There was no significant improvement in overall intelligence as seen in the Ravens progressive matrices between the experimental and control groups.

Conclusion: The study shows that the multiple micronutrient fortified salt is effective in improving multiple micronutrient status and cognition in children.

Key Words: child nutrition, India, multiple micronutrients, salt fortification, iron, vitamin A, B complex vitamins, cognition

INTRODUCTION

In developing countries multiple micronutrient deficiencies occur in the poorer segment of the population.^{1,2} However the approach to combat micronutrient deficiencies has been to tackle individual micronutrients, for example supply of iron and folic acid tablets to pregnant women, vitamin A drops to children etc. There is a need to tackle multiple micronutrient deficiencies in unison and not singly.³ Thus, a multiple micronutrient fortified salt was developed which contained vitamin A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate, iron and iodine. If used in cooking it would supply all these nutrients to the entire family everyday. We have tested the bioefficacy of this fortified salt and its effect on cognition in school children in this study.

The iron source used in earlier studies were normally inorganic compounds.⁴⁻⁷ Since the Indian diet is high in phytates which inhibit iron absorption, it was felt that the iron source has to be bioavailable despite the presence of phytates and one way of doing this is to use highly bioavailable compounds like ferrous sulphate and the other

is to give the iron source in a chelated form. We have done both in this study and have used chelated ferrous sulphate as the iron source and tested its bioefficacy in children and also seen the effect on cognition in school children.

MATERIALS AND METHODS

Bioefficacy Study

Subjects. The Research Design was a pre- post test design with experimental (N=63) and control (N=66) groups. The children residing in the residential school constituted the experimental group. The children who lived in communities nearby and attended the day school constituted the control group.

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There was no intervention in the children belonging to the control group except deworming. A survey was conducted on different schools and the school was chosen where there were minimum instances of outside cooked food (unfortified) served to the children and where there were minimum intervening holidays when the children would go home and cause a disruption in the study.

The experimental and control groups of children were similar in age, intelligence, nutrient intake and socioeconomic status (monthly family income of about Rs1500 (US\$ 30)). Analysis of the results of the intelligence tests showed that there was no statistical difference between the experimental and control groups with reference to the baseline scores of the Ravens progressive matrices showing that the experimental and control groups were comparable with reference to intelligence at baseline and hence can be compared for cognitive outcomes. The mean baseline score of Ravens progressive matrices in the experimental group was 34.7 ± 29.5 and the mean baseline score of the control group was 26.8 ± 26.9 and there was no significant difference ($p < 0.05$) in the scores of the experimental and control group.

Dietary assessment of the households of a sub-sample of control group (N=25) showed that there were no significant differences in the nutrient intake of both the experimental and control groups. The dietary consumption was calculated by assessing the details of food consumed in the past 3 days in the sub-sample of the control group households and by assessing the consumption details from the stock register in the experimental group.

The study was approved by the Institutional Ethics committee also referred to as the Institutional Review Board of Sundar Serendipity Foundation and the Doctoral committee of M.S.Swaminathan Research Foundation. Informed written consent was obtained from the School Director and informed oral consent was obtained from the parents/legal guardians of all the children. The parents/legal guardians of the children in the residential school were informed about the use of the fortified salt in the cooking of all the meals in the schools, blood tests to be performed and periodic de-worming. The parents/legal guardians of the day scholars (control group) were informed about the periodic de-worming and that blood tests would be done in all the children for assessment of anaemia and the children with severe anaemia (Hb less than 8g/dL) would be treated for the anaemia immediately therapeutically. These children were excluded from the study. All the children who were anaemic at the end of the study were treated with ferrous sulphate tablets with 60mg elemental iron for 3 months.

Study design. The fortified salt was used in all the meals cooked for the children in the experimental group for a period of one year. There was no intervention in the control group except for de-worming. De-worming was done in both experimental and control children by giving a tablet of albendazole 400 mgs at baseline, after 6 months of intervention and after one year of intervention (end of study). De-worming was done to ensure that there are no worms which compete for the micronutrients and ensures the intestinal tract is clear for bioabsorption of the micronutrients as in other studies.^{8,9}

The fortified salt was supplied to the school every month and the continuous use of the fortified salt in all the meals prepared everyday was monitored. The period of the study was one year.

The fortified salt did not change the color or taste of any food preparation. The average consumption of salt was 10 g per child per day and the children of the experimental group consumed 3 meals per day and an afternoon snack, and all the food was prepared with fortified salt. It was observed that generally there was no wastage of food prepared in the residential school. All the food prepared is consumed.

The bio-chemical parameters that were estimated for all the children were hemoglobin, hematocrit, red blood cell count, urinary iodine and serum vitamin A. Hemoglobin estimation was carried out before the start of the study, six months after the commencement and one year after commencement (end) of the study. The hematocrit, red blood cell count, urinary iodine and serum vitamin A were done before the study began (base line) and at the end of one year of the study (endline).

Blood collection, storage and laboratory analysis. 3 mL of venous blood was drawn from each child. Five hundred microlitres of blood was transferred into vials which had EDTA as anticoagulant. Hemoglobin, hematocrit and red cell counts were estimated in this sample, within a few hours of blood collection. The blood samples were transferred to the laboratory within 2 hours of collection at the school. The test for serum vitamin A were carried out in the remaining blood sample, transferred into vials wrapped in black paper to prevent sun exposure. When the blood was clotted, serum was separated from this sample and frozen within a few hours of blood collection at -20 degrees Celsius. Analysis of serum vitamin A was completed within a month after blood collection. The samples were processed in a dark room with yellow lighting to prevent retinol isomerization.

Hemoglobin was estimated by cyanamethemoglobin method.¹⁰ Hematocrit was estimated by centrifuging blood in wintrobe tubes.¹⁰ Red blood cell count was done by counting the cells using the neubauer counting chamber.¹⁰ Serum Vitamin A was estimated by the Carrprice method.¹¹ using a spectrophotometer (UV double beam Shimadzu spectrophotometer). Urinary iodine was measured by using the Sandell-Kolthoff reaction as modified by Pino *et al.*¹² Hemoglobin was done in duplicate for all the samples. In hematocrit, RBC count, serum vitamin A and urinary iodine, in 10% of the samples the test was done twice for validation.

Dosage of Iron. Most of the studies reviewed in literature gave iron in the form of ferrous sulphate tablets for periods ranging from 2 to 8 months.¹³⁻¹⁶ Our study is different in that the experimental group children received 10 mg of chelated elemental iron every day through the multiple micro nutrient fortified salt for a period of one year. The iron was in a chelated form with the biopromotor added. Chelated iron compounds have a much higher bioavailability than inorganic iron compounds. Ferrous sulphate monohydrate was chelated by us in our laboratory with chelating agents and the absorption promoter

was further added to enhance the iron absorption. Ferrous Sulphate was chelated with malic acid and sodium hexameta phosphate. The acidic pH was maintained by sodium dihydrogen phosphate which served as an absorption promoter. The resultant chelated iron complex was white in color.

Statistics. Statistical analysis was done using SPSS 11.0 (SPSS Inc., Chicago IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle WA, USA). ANOVA was done to compare the effects of the nutritional intervention and cognition between groups and over time. Mann-Whitney test and Wilcoxon signed rank test was used to test the significance of median urinary iodine analysis.

Tests for Memory, Concentration and IQ

Iron deficiency anemia is one of the important causes of lowered concentration abilities and impaired memory skills. The present study involves giving a battery of memory tests and concentration tests to see if there is an improvement in these memory and concentration abilities when anemia is reduced through the nutritive intervention.

To test the memory in children we used the Children's memory test developed by NIMHANS (National institute of mental health and neurological sciences, Bangalore, India). Though the NIMHANS memory test had verbal and nonverbal components, the children we were dealing with are rural children who studied in the regional language and not in English. The verbal component of the NIMHANS memory test was in English. We therefore chose to give the children only the nonverbal component of the test where English language was not a barrier. This was possible because each test was individually scored. The individual scoring also helps us find out in which aspects of memory does improvement take place when iron and other micronutrients are given.

In both the experimental and control groups, a battery of tests developed and standardized on Indian children to suit Indian conditions by NIMHANS to assess memory and concentration was administered. The children in both the experimental and control groups were also administered the Ravens Children's Progressive Matrices, a test to assess the IQ of these children and a letter cancellation test to assess concentration. These tests were administered before the start of the study (base line) and after one year of nutrient intervention. There are very few children in both the experimental and control groups whose hemoglobin is more than 12 g/dL. Therefore this study has no non-anemic controls.

Description of the memory tests

Personal information. This test is a measure of remote memory which constitutes recall of past events of personal life. This is adopted from Wechsler memory scale and PGI memory scale.

Digit span. This subtest is taken from Wechsler memory scale. This comprises of span for digits forward and backward. The maximum number of digits used in the series is limited to 9. This test is a measure of attention and concentration.

Delayed response learning. This essentially requires the ability to delay the previous response in order to arrive at a final solution. This measures delayed memory span. There are 4 sets of fairly simple arithmetical problems. Each problem consists of 2 parts presented one after the other. In the first part a simple arithmetical problem is given, the child solves it and keeps the result in mind and then solves the second part of the problem 10 seconds later incorporating the result from the previous part.

Mann-Suiter Visual memory screen for objects (picture recall test). This is designed to assess the ability to visualise pictures of common objects presented in groups. There are 4 cards. On the first card there are 2 pictures and it was exposed for 2 seconds. The second card has 3 pictures and it was exposed for 3 seconds, the 3rd card had 4 pictures and the 4th card had 5 pictures and it was exposed for 4 seconds and 5 seconds respectively. The child was expected to recall the pictures in the same sequence. This test measures short term visual memory.

Benton Visual Retention Test (BVRT). This test is designed to assess visual perception, Visual memory and visual-constructive abilities. There are 10 cards. Each card is exposed for 10 seconds and the child is asked to reproduce the design from memory. This test measures the visual spatial perception, visual and verbal conceptualization and immediate memory span.

Cattells retentivity test. It consists of complex and unfamiliar designs of irregular geometric figures which cannot elicit any verbal associations. On a card 10 geometrical figures are presented for 30 seconds, after a 2 minute pause and from the second card the child has to recognize the geometrical figures which he has already seen in the first card. This measures the visual recall for irregular geometrical designs and delayed memory span.

Letter cancellation test

This test is a measure of concentration. The children are given the test which has many alphabets typed out in rows and the children are instructed to score out the A's and E's within a period of 2 minutes. If the child has omitted to score a letter or if he/she has scored a letter which is not A or E, it is considered as a wrong. If the child has correctly struck out an A or E it is considered as

Table 1. Composition of the multiple micronutrient fortified salt

| Ingredient | Nutrient status |
|----------------------------|-----------------|
| Vitamin A IU/g | 300 |
| Vitamin B2 mg/kg | 200 |
| Calcium pantothenate mg/kg | 200 |
| Niacin g/kg | 3 |
| Vitamin B1 mg/kg | 200 |
| Vitamin B6 mg/kg | 200 |
| Folic Acid mg/kg | 5 |
| Vitamin B12 mcg/kg | 400 |
| Iron ppm | 1000 |
| Iodine ppm | 40 |

right. The final score is obtained by subtracting the total of wrongs from the totals of rights.

Ravens coloured progressive matrices

This is an IQ test to measure intelligence in children. The same tests were administered before the intervention program and repeated after one year of nutritive intervention.

Scoring for cognitive studies

Based on the age of the child the raw score is converted to the percentage score, as per the standardization done by NIMHANS on Indian children. Therefore age matching between the experimental and control group is taken care of in the scoring itself. The score obtained at the end of the study is subtracted from the initial score to get the increment in score. The comparison is made between the experiment and control with regard to the increment in scores to offset the increment due to familiarity in the retest.

In earlier studies in India,¹⁷ it was seen that there were increment in scores in arithmetic and digit span subtests in the placebo group also. In a Thailand study,¹⁸ all the children improved their scores at follow-up regardless of their iron status. It can be reasoned out that there is always a familiarity element when a retest is given and this familiarity leads to improvement in scores in the control also. To offset this improvement in scores due to familiarity, the endline score is subtracted from baseline score and the increment in scores is taken to consider whether there is an improvement of the experimental group over the control.

RESULTS

Efficacy study

It can be seen that there was a significant ($p<0.05$) improvement in the experimental group in hemoglobin, red cell count, serum vitamin A and urinary iodine whereas in the control group there was a statistically significant decline ($p<0.05$) in hemoglobin, hematocrit, red cell count and urinary iodine. (See table 2).

Angular stomatitis

In the experimental group the prevalence of angular

stomatitis due to vitamin B complex deficiencies was 30.4% at the start of the study, was totally eliminated within 2 months of the start of the study and did not reappear throughout the whole study period. In the control group the prevalence of angular stomatitis was 3.25% at the start of the study and increased to 25.5% at the end of the study (see Table 2).

Cognitive study

We find that out of the 7 memory tests administered, in 4 of the tests, namely the Benton Visual Retention Test, the Cattells retentivity test, Mann-Suiter Visual memory screen for objects (picture recall test) and Delayed Response Learning test, the mean increment in scores in the experimental group is significantly more ($p<0.05$) than the control group. Only in the personal information test and the digit forward and backward test, the increment in scores in the experimental group is not statistically significant. In the letter cancellation test which is a measure of attention and concentration too the mean increment in score in the experimental group is significantly more than ($p<0.05$) the control. There are no significant differences with respect to the intelligence test - the Raven's coloured progressive matrices in the experimental and control group. This is understandable as there usually will not be an improvement in the overall intelligence but only in certain specific areas of memory which is measured by the memory tests. (see table 3).

DISCUSSION

Many studies have shown that in school aged children, initially lowered test scores of cognition can be improved when their hemoglobin increases when anaemia is alleviated.¹⁹⁻²² This may be because of a large number of placebo controlled trials, which are able to pick up treatment effects and the increased sensitivity of the tests used. It may also be because the effects of iron deficiency in school aged children are more transitory than in infants and are thus more responsive to the effects of iron treatment. Most of the studies showed significant improvement in cognitive function or educational achievement of the children who received iron supplements (iron alone or iron and folic acid) compared to those who received the

Table 2. Biochemical and Clinical parameters at baseline and at the end of the study

| | experimental group N=63 | | control group N=66 | |
|------------------------------|----------------------------|------------------------------|---------------------------|----------------------------|
| | Base line | End point | Base line | End point |
| Hemoglobin gms/dL* | 9.55 ± 1.21 ^{ac} | 10.2 ± 0.77 ^a | 10.4 ± 0.59 ^{bc} | 10.1 ± 0.75 ^b |
| Hematocrit L/L* | 0.32 ± 0.03 | 0.32 ± 0.03 ^d | 0.31 ± 0.02 ^b | 0.29 ± 0.02 ^{bd} |
| Red blood cells* million/cmm | 3.47 ± 0.24 ^{ac} | 3.97 ± 0.39 ^{ad} | 3.80 ± 0.48 ^{bc} | 3.48 ± 0.26 ^{bd} |
| Serum vitamin A [µg/dL] * | 36.5 ± 12.5 ^{ac} | 41.4 ± 14.8 ^a | 42.7 ± 12.8 ^c | 46.2 ± 18.6 |
| Urinary iodine [µg/L] ** | 245 (100-600) ^a | 510 (125-600) ^{a d} | 230 (55-600) ^b | 70 (70-635) ^{b d} |
| Angular stomatitis [%] | 30.4 | 0 | 3.25 | 25.5 |

^a: Significant increase ($p<0.05$) from baseline to endpoint in the experimental group. ^b: Significant decrease ($p<0.05$) from baseline to endpoint in the control group. ^c Values differ significantly ($p<0.05$) at baseline between experimental and control groups. ^d Experimental group significantly higher ($p<0.05$) than control group at endpoint. *Data given as mean ± SD. ** Data given as median (range) Mann-Whitney test and Wilcoxon signed rank test used to test the significance.

Table 3. Cognitive tests: Increment in Scores

| Name of the test | Test measures | Experiment n=63 | Control n=66 |
|---|-----------------------------|--------------------------|--------------------------|
| Benton Visual Retention Test (BVRT) | memory | 15.5 ± 20.9 ^a | 8.48 ± 19.4 ^a |
| Cattells retentivity test. | memory | 8.73 ± 19.2 ^a | 2.57 ± 16.1 ^a |
| Mann-Suiter Visual memory screen for objects (picture recall test) | memory | 21.2 ± 29.6 ^a | 7.27 ± 16.1 ^a |
| Delayed Response Learning test | memory | 22.2 ± 33.7 ^a | 5.90 ± 20.4 ^a |
| Personal Information test | memory | 31.6 ± 42.5 | 24.7 ± 35.8 |
| Digit Forward test | memory | 0.95 ± 11.0 | 1.6 ± 12.5 |
| Digit Backward test | memory | 2.38 ± 21.8 | 4.24 ± 19.1 |
| Letter cancellation test | Attention and concentration | 4.70 ± 8.43 ^a | 1.53 ± 8.83 ^a |
| Ravens coloured progressive matrices | intelligence | 5 ± 21.7 | 4.6 ± 18.8 |

^a: significant improvement of the experimental group ($p < 0.05$) over the control. Data given as mean ± SD

placebo.^{6,17} In contrast, it has been observed that in infants, the reduced cognitive scores in anaemic infants does not change or improve when the anaemia is alleviated. The adverse effects on cognitive and educational test performance due to iron deficiency anemia in preschool and school aged children appear more transitory in nature than the effects on development on infants and imply that treatment of iron deficiency anemia in preschool and school aged children through iron supplementation programmes may be beneficial and have immediate effects. Anemia causes poor attentiveness, poor memory and poor academic performance in school age children. Anaemic infants are often irritable, restless and show behavioral abnormalities like lack of attention, fatigue and insecurity. Poor attention span, memory and concentration as well as concept acquisition leading to poor school performance have been attributed to anemia during this phase of critical learning.

In our study we find that when the fortified salt is used for a period of one year, it has resulted in a significant improvement ($p < 0.05$) in hemoglobin, red cell count, serum vitamin A and urinary iodine in the experimental group. This shows the bioavailability of vitamin A, iron and iodine. The bioavailability of the B complex vitamins, especially vitamin B₂ is seen by the disappearance of angular stomatitis in the experimental group. The biochemical analysis of all the other nutrients present in salt was not done.

There is a significant decrease ($p < 0.05$) in hemoglobin, hematocrit, red cell count and urinary iodine in the control group. This decrease in the iron status in the control group of children has been observed in other studies.^{23,24} The drop in urinary iodine values in the control group may be due to the interruption of using iodised salt by families who were previously consuming it. This issue was brought to the notice of the communities and people were advised to consume iodised salt only.

When the cognitive tests are administered, we find that in 4 memory tests out of 7 tests administered, and in the letter cancellation test which measures attention and concentration, we find that the increment in scores in the experimental group is significantly higher ($p < 0.05$) than the control. In the personal information test and digit forward and digit backward tests, no significant improvements were observed in the experimental group. This may be because the digit forward and digit backward tests in-

involved testing the mathematical ability to remember numbers in a forward and backward sequence. This area of their memory has apparently not improved by the increase in iron status in the experimental group. Thus the improvement in iron, vitamin A and urinary iodine through the use of the multiple micronutrient fortified cooking salt has resulted in concomitant increase in the cognitive parameters in the experimental group of children.

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Original Article

Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children

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食鹽添加多種微量營養素對 Chennai 學童認知的影響

目的：測試添加多種微量營養素的食鹽對改善學童微量營養素狀況及健康的效力，與其對認知的影響。

方法：食鹽添加多種微量營養素，包含有螯合硫化鐵、微囊化維生素 A、B1、B2、B6、B12、葉酸、菸鹼酸、泛酸鈣及碘。以印度 Chennai 7-11 歲學童評估該強化添加食鹽的效力。實驗組(N=63)，學校廚房採用強化食鹽烹調食物為期一年。控制組(N=66)，為沒有在學校飲食者。研究開始及一年研究結束後，測量血紅素、紅血球計數、血比容、血清維生素 A、尿碘及口角炎盛行率。所有兒童在研究開始及結束時均實施一套 7 項記憶力測驗（個人常識測驗、Mann-Suiter 視覺物體記憶力篩選、數字順背記憶測驗、數字倒背記憶測驗、延遲反應測驗、班頓視覺保留測驗以及 Cattells 記憶力持久測驗）、一項專注力測驗（刪字測驗）及一項針對智力的測驗（瑞文氏智力測驗）。

結果：實驗組在血紅素、紅血球、尿碘及血清維生素 A 有顯著的改善 ($p<0.05$)，反之控制組在血紅素，血比容，紅血球及尿碘則有統計上顯著下降 ($p<0.05$)。實驗組口角炎較研究開始降低 30.4%，反之控制組由 3.25% 增加至 25.5%。在 7 項記憶力測試中有 4 項以及專注力的刪字測驗，實驗組平均增加的分數顯著的較控制組高 ($p<0.05$)。以瑞文氏智力測驗評估整體智力的改善，實驗組與對照組沒有顯著差異。

結論：本研究顯示添加多種微量營養素的食鹽，可以有效改善兒童的多種微量營養素狀況及認知。

關鍵字：兒童營養、印度、多種微量營養素、食鹽強化、鐵、維生素 A、維生素 B 群、認知。