Efficacy of fortification of school meals with ferrous glycine phosphate and riboflavin against anemia and angular stomatitis in schoolchildren

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Abstract

Objective. *Multiple micronutrient deficiencies exist in many developing countries. We conducted a study to test the efficacy of ferrous glycine phosphate in reducing anemia and of riboflavin in reducing angular stomatitis when these micronutrients were added to the noon meal for schoolchildren.*

Methods. *A pre- and post-test design was used to study children 5 to 9 years of age, with an experimental and a control group. Two schools in the same locality in Chennai were chosen for the study. The experimental school had 65 children and the control school had 71 children, all of whom consumed a noon meal at the school daily. The children in the experimental school received a powder containing ferrous glycine phosphate and riboflavin, which was added to the meal during cooking every day for 6 months. The dosage was 28 mg of elemental iron and 1 mg of riboflavin per child per day. The children attended school for 5 days each week from Monday to Friday, except for holidays; they received the fortificants on 100 days during the 6-month period. There was no intervention in the control school. Children in the experimental and control groups were matched by socioeconomic status, age, and eating habits at baseline. All the children in the experimental and control schools were dewormed at baseline and at endline after 6 months. Hemoglobin was measured by the cynamethemoglobin method at baseline and endline.*

Results. *Binary logistic regression showed a significant ($p < .001$) time x group interaction for anemia. The prevalence of anemia in the experimental school was 69.0% at baseline and 32.8% after 100 days of intervention over 6 months, a statistically significant change ($p < .001$). The prevalence of anemia in the control school was 91.5% at baseline and increased to 97.2% at endline; the increase was not statistically significant. The prevalence of angular stomatitis was reduced from 21% at baseline to 0% at endline in the experimental school, whereas it was 23% at baseline and 20% at endline in the control school.*

Conclusions. *The added fortificants reduced the prevalence of the micronutrient deficiencies.*

Key words: Anemia, children, ferrous glycine phosphate, fortificant

Introduction

Micronutrient deficiencies in developing countries are a consequence of the plant-based cereal diets typically consumed in these areas [1, 2]. Dietary phytate inhibits the absorption of many micronutrients, notably iron and zinc. Micronutrient deficiencies in infancy can cause impairments of physical development and cognition that may be irreversible [3–5]. Iron and iodine deficiencies affect more than 30% of the global population [6]. It is estimated that iron-deficiency anemia affects nearly 1 billion people worldwide [7]. It has been suggested that supplementation with multiple micronutrients may be the best way to improve the nutritional status of malnourished populations [8].

In Tamilnadu, India, the government provides a daily noon meal of rice with lentils and vegetables to children studying in government and government-affiliated schools. Each child also receives two eggs a week. The noon meal scheme was ideal for the delivery of additional micronutrients. This study tested the stability of ferrous glycine phosphate during storage and tested the bioavailability of ferrous glycine phosphate and riboflavin in the target group of schoolchildren.

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Methods

Subjects

The study had a pre- and post-test design, with experimental and control groups. The two schools designated as the experimental and control schools were randomly selected from a list of schools in Chennai that provided the noon meal. After the two schools had been selected, we verified that the children in the schools were matched for age, dietary patterns, and socioeconomic status. The experimental school was then provided with a powder containing riboflavin and ferrous glycine phosphate.

For ethical reasons, it was decided that children with severe anemia (hemoglobin < 8 g/dL) in both the experimental and the control groups would be treated with ferrous sulfate tablets for 3 months and excluded from the study. The children in both groups were dewormed at baseline and endline. The study was conducted from July to December 2005. The experimental and control groups of children were homogeneous in terms of age and socioeconomic status; the families of all the children had a monthly income of less than Rs2000 (US$50).

Sample size

We chose an alpha of .05 and a power of 80% with a two-tailed test for calculations of sample size. In our earlier studies [9] on the use of chelated ferrous sulfate in multiple-micronutrient-fortified salt for schoolchildren 5 to 15 years of age, we found an increase in hemoglobin of 0.5 g/dL with a standard deviation of 1. The use of similar assumptions for this study yielded a required sample size of 63 children in each group.

Manufacture of the fortificants

Ferrous glycine sulfate was chelated with phosphoric acid to yield ferrous glycine phosphate. The fortificants ferrous glycine phosphate and riboflavin were mixed in a ribbon blender (Bhuvaneshwari Engineering, Chennai, India) at 50 rpm. The homogeneity of the powdered micronutrient contents was established at the manufacturing stage by assessing the micronutrient contents in different parts of the blender. It was determined that all of the micronutrients were uniformly and homogeneously distributed within the product.

Dosage of micronutrients

The powder was added to the noon meal prepared for the children 5 days a week. Since the number of children eating the noon meal was fixed, we measured the quantities so that each packet provided a dosage of 28 mg of elemental iron from ferrous glycine phosphate and 1 mg of riboflavin per child per day. One packet was added to the food during cooking each day. The monthly requirement was delivered to the school, and the packets were counted at the end of the month to verify that their contents had been added to the food on each school day.

The cooking staff of the experimental school certified that the micronutrient powder did not change the color or taste of the food. Each school has a central kitchen where the food is prepared and a central dining room where the children eat. It was generally observed that there was no wastage of the food prepared in the schools; all prepared food was consumed. The children were served the quantity of food required by them, and there was no food left over on the plate.

In both the schools, the average attendance of the children during the 6 months of the study was more than 90%.

Blood collection and laboratory analysis

Samples of venous blood (1 mL) were drawn from each child at the school and transferred into vials with ethylenediaminetetraacetate (EDTA) as an anticoagulant. The hemoglobin measurements were performed on these samples in the laboratory within a few hours after blood collection. The blood samples were transferred to the laboratory within 2 hours of collection. Hemoglobin was measured by the cyanmethemoglobin method [10] with a spectrophotometer (UV double-beam model, Shimadzu, Japan) at baseline and after 6 months at endline. Measurements were performed in duplicate in all the samples, and the analysis was repeated if the results differed by more than 5%. Anemia in children aged 5 to 11 years is defined as a hemoglobin level less than 11.5 g/dL [8]. Since the children in this study were 5 to 9 years of age, 11.5 g/dL was used as the cutoff point for defining anemia.

Deworming

Both the experimental and the control children were given a tablet of albendazole (400 mg) at baseline and endline. 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 [11, 12].

Clinical assessment

Clinical assessment of angular stomatitis, a condition caused by deficiencies of B-complex vitamins, was conducted by a physician at baseline and endline.

Statistical analysis

Statistical analysis was performed with SPSS, version 11.0, and Microsoft Excel 2000. Repeated-measures analysis of variance was used to compare the effects of group x time for hemoglobin. If the interaction effect of group x time was significant ($p < .05$), t-tests between groups and paired t-tests within groups were performed. Proportions were compared by chi-squared tests. Binary logistic regression was performed to compare the effects of group x time for the binary variable of anemia. Significance was set at $p < .05$.

Ethical issues

The study was approved by the institutional review board of the Sundar Serendipity Foundation. Informed written consent was obtained from the school directors, and informed written consent was obtained from the parents or legal guardians of all of the children. The parents of the children in the experimental school were informed about the use of the micronutrient powder in the noon meals cooked in the schools and about the blood tests to be performed. The parents of the children in the control school were informed that blood tests would be performed on all of the children and that those children with severe anemia (hemoglobin < 8 g/dL) would be treated with ferrous sulfate tablets. It was decided that anemic children in both the experimental and the control groups would be treated with ferrous sulfate tablets (60 mg elemental iron) after the end of the study for a period of 3 months.

Results

Stability study

The stability of ferrous glycine phosphate and riboflavin for 12 months at 30°C and 45% relative humidity was measured. The stability of riboflavin and ferrous glycine phosphate during cooking at 95°C for 20 minutes was also estimated. The results are given in **table 1**. Both ferrous glycine phosphate and riboflavin were very stable during cooking and storage.

Efficacy study

Sixty-five children in the experimental school and 71 children in the control school completed the study. Four children in the experimental school and three children in the control school were absent when endline blood samples were taken and hence were not included in the statistical analysis. Only one child in the experimental group had a baseline hemoglobin level less than 8 g/dL; she was given ferrous sulfate tablets for 3 months and excluded from the study for ethical reasons.

At baseline, the mean hemoglobin level was significantly higher in the experimental group than in the control group (11.02 and 10.68 g/dL, respectively; $p < .05$), despite the fact that the children in both groups belonged to the same socioeconomic group, had similar dietary habits, and lived in the same community. At endline, the hemoglobin level had increased to 11.71 g/dL in the experimental group, a statistically significant change ($p < .001$), and decreased to 10.40 g/dL in the control group, which was also a significant change ($p < .001$) (**table 2**). The prevalence of anemia (hemoglobin < 11.5 g/dL) at baseline was 69.0% in the experimental group and 91.5% in the control group. At 6 months, after 100 days of intervention, the prevalence of anemia in the experimental group had decreased to 32.8%, a statistically significant change ($p < .001$); in the control group, the prevalence had increased to 97.2%, although this increase was not statistically significant (**table 3**). At baseline, 21% of the children in the experimental group and 23% of the children in the control group had angular stomatitis. At the end of the study, angular stomatitis had completely disappeared in the experimental group, [a statistically significant change (p < 0.001)] and had decreased in prevalence to 20% in the control school, though this change had no statistical significance.

Discussion

There are many iron fortificants available in the market. Some fortificants, such as ferrous sulfate, have high bioavailability, but they have several problems associated with fortification, such as discoloration of the food

TABLE 1. Stability of ferrous glycine phosphate and riboflavin during cooking and storage

Ingredient	Initial amount	Amount after cooking[a]	Loss after cooking	Amount after 1 yr storage	Loss after 1 yr storage
Iron in ferrous glycine phosphate (elemental iron mg per gram of ferrous glycine phosphate)	183 mg elemental iron per gram of ferrous glycine phosphate	183 mg elemental iron per gram of ferrous glycine phosphate	0%	182 mg elemental iron per gram of ferrous glycine phosphate	0.55%
Riboflavin	1 mg	0.997 mg	0.3%	0.985 mg	1.5%

a. Cooking is done for 20 min at 95°C.

during cooking or imparting of a distinct metallic iron taste to the food. Other fortificants, such as carbonyl iron, electrolytic iron, or ferric pyrophosphate, have lower bioavailability but do not cause coloration problems when added to food products. We decided to chelate ferrous glycine sulfate with phosphoric acid to produce ferrous glycine phosphate, which has a high bioavailability but does not have any organoleptic problems and does not discolor or impart any iron taste to the food during cooking. Phosphoric acid, in this case, acts as a sequestering agent that prevents the adverse effects of the oxidative breakdown of foods catalyzed by iron and thus averts discoloration or imparting of taste or rancidity to foods during cooking. Since ferrous glycine phosphate did not impart any taste or smell or change the color of the food during cooking, it was decided to administer 1 Recommended Dietary Allowance (RDA) of iron per child per day and test the bioavailability in a relatively shorter period of 100 days of intervention, rather than the more standard 6+ months of most fortification studies.

This study was undertaken to examine the feasibility of adding micronutrient fortificants to a noonday rice meal during cooking. Rice is the staple cereal in South India, whereas wheat is the staple cereal in North India. Fortification of wheat flour with iron and folic acid is feasible and has been tried in many states in North India, but fortification of rice has remained a challenge. This study was therefore undertaken to find out the feasibility of adding the fortificants at the cooking stage itself in noon meal centers in Tamilnadu, where the government provides a rice-based noon meal to all children attending government and government-aided schools. If successful, this method would be a way to deliver the micronutrients through the cooked rice meal, as an alternative to fortification of rice. In our earlier studies [13, 14], we showed that delivery of multiple micronutrients by a multiple-micronutrient food supplement (a powder with multiple micronutrients added to food during cooking) was feasible. However in those studies, the children were residential schoolchildren who received the supplement containing 1 RDA of multiple micronutrients in all their meals (4 meals) throughout the day for a period ranging from 9 to 12 months. In the present study, micronutrients had to be delivered in a single noon meal. We therefore devised a strategy of delivering a rather larger dose of approximately 1 RDA [15] of iron (28 mg) and riboflavin (1 mg) 5 days a week for a short period of 100 days of intervention. (In the previous studies, the 1RDA was delivered through 3 meals and an afternoon snack (4 meals). In the current study 1RDA had to be delivered through just one meal.) The RDAs of iron and riboflavin for Indian children 7 to 9 years of age are 26 mg/day and 1.2 mg/day, respectively [15].

We included riboflavin because of the presence of angular stomatitis at baseline in 21% of the children in the experimental group and 23% of the children in the control group. Riboflavin supplementation has been shown to improve hemoglobin in earlier studies [16]. It has been shown that riboflavin has a direct role in the release of iron from ferritin [17, 18]. Animal studies have shown that riboflavin deficiency impairs iron absorption [19, 20]. Moreover, other studies have shown [21–24] better hematological response when riboflavin was given along with iron than when iron was given alone. A recent study in Bangalore, a neighboring state, on 100 nonpregnant, nonlactating women [25] showed good correlations between blood hemoglobin and serum ferritin and dietary intakes of riboflavin. We felt that if the high prevalence of angular stomatitis was due to riboflavin deficiency, then iron absorption might be impaired if iron alone was given as a fortificant. Therefore we included both riboflavin and iron as fortificants in this study.

Angular stomatitis may be due to infection when it responds to topical applications of antibiotics or gentian violet, as in earlier studies [26], or it may be due to micronutrient deficiencies [27]. Since angular stomatitis disappeared when riboflavin was used as a fortificant in cooking, it may be concluded that the cause of angular stomatitis in our study was micronutrient deficiency and not infection.

The bioavailability of vitamins and minerals given in the form of supplements as tablets or syrups has

TABLE 2. Hemoglobin levels in the experimental and control groups at baseline and at endline after 6 months (mean ± SD g/dL)

Time	Experimental group (n = 65)	Control group (n = 71)
Baseline	11.02 ± 1.03^{ad}	10.68 ± 0.5^{bd}
Endline	11.71 ± 0.94^{ac}	10.40 ± 0.49^{bc}

There was a significant group x time interaction, $p < .001$ (ANOVA repeated measures) for hemoglobin.

a. Significant increase $p < .001$ from baseline to endline in the experimental group.

b. Significant decrease $p < .001$ from baseline to endline in the control group.

c. Hemoglobin level was significantly greater in the experimental than in the control group at endline.

d. Hemoglobin level was significantly greater in the experimental than in the control group at baseline.

TABLE 3. Prevalence of anemia in the experimental and control groups at baseline and at endline after 6 months

Time	Experimental group (n = 65)	Control group (n = 71)
Baseline	69.0^{a}	91.5^{b}
Endline	32.8^{a}	97.2^{b}

There was a significant group x time interaction, $p < .001$ (binary logistic regression) for anemia.

a. Significant decrease ($p < .001$) in prevalence of anemia in the experimental group.

b. No significant change in prevalence of anemia in the control group.

been extensively studied. A difference of our study was that the vitamins and mineral used as fortificants had to withstand the high temperatures of cooking and be stable during storage. We find that both ferrous glycine phosphate and riboflavin were extremely stable during cooking and storage and were able to improve hemoglobin status and eliminate angular stomatitis, respectively; hence, they were bioavailable.

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