

EFFECTIVENESS OF IRON FORTIFIED MILK (NaFeEDTA) IN AN INTERVENTION PROJECT EMPHASIZING ON IRON STATUS OF SCHOOL AGED CHILDREN IN KHON KAEN PROVINCE, THAILAND.

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ABSTRACT

The effectiveness of iron fortified milk (NaFeEDTA) on the iron status and the prevalence of anemia of school children were studied in Khon Kaen province, Thailand. The milk supplemented with 5 mg iron per sachet per day was administered to 160 school children at age ranging from 6 - 14 years old. One hundred and sixty school aged children were participated and divided into two groups. One group was allowed to consume fortified milk while the second group was allowed to consume non-fortified milk. A sachet of milk was served daily five days per week with 200 ml of milk containing 5 mg of iron (Fe), which designated as NaFeEDTA, for three months. The levels of hemoglobin, hematocrit, serum ferritin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were analyzed from blood samples taken before and three months after the intervention. The prevalence of anemia was measured at baseline and three months to after the intervention. There were statistical differences in the changes of hemoglobin concentration, hematocrit, mean cell volumes, and mean corpuscular hemoglobin between fortified milk, and non-fortified milk group at significant level, $p < 0.05$, after three months of intervention. There was no statistical difference in serum ferritin, mean corpuscular hemoglobin concentration, and the prevalence of anemia change after intervention. From dietary assessment, overall daily intake provided adequate energy, fat, protein, with the exception of iron, calcium, vitamin A, and vitamin C, which were iron absorption enhancers for both groups when compared with the percentage of Thai Dietary Reference Intake (DRI). This study indicated that NaFeEDTA fortified milk has the potential to improve the hematological status of school children who received iron fortified milk.

KeyWords: Iron, food fortification, iron deficiency anemia, nutrition, iron fortified milk.

INTRODUCTION

Anemia is a major problem among woman and young children, but there is growing evidence that it is also a problem among school-aged children. Information from a National Food and Nutrition Survey conducted in Thailand around the year 2000 indicated that the prevalence of anemia in school-age children (6-14 years of age) increased to 13.1 % by using hematocrit values as criteria and to 26.7% when using hemoglobin as criteria. In Khon Kaen province in the northeastern part of Thailand, anemia prevalence was as high as 14.3% when using the level of hematocrit, and 42.8% for those children that determined by hemoglobin (UNSCN, 2006). Iron deficiency anemia among school children had been assessed on district level at the Khon Kaen province and had been documented at the school health report of 2005 by the Si Chomphu Hospital, Si Chomphu district, Khon Kaen province. It was found that the prevalence of iron deficiency anemia in school children at the Primary level was as high as 21.3% (Institute of Provincial Public Health, 2005). Presently, the Royal Thai government tries to solve nutritional problems in school children by introducing school milk programs. Milk is rich in high quality protein (FNRI, 2006), and providing energy as well as other micronutrients and can serve as vehicle for iron fortification. Iron salts, such as ferrous sulphate and fumarate, are reasonably well absorbed and are suitable for fortification when the storage of the fortified food is not too long. The iron salt fortificants, NaFeEDTA, has the potential to be used as iron fortification in the future when compared with the one that commonly used. The rate of NaFeEDTA absorption is two to three times higher than that of ferrous sulphate. Its structure affords some protection against phytate inhibition of iron absorption. It is about one third as well absorbed as ferrous sulphate, but has the advantages of greater stability and of increasing the absorption of native non-hemoglobin iron and zinc in food

(Seshadri and Gopaldas, 1989). The Royal Thai government will continue to support the school milk program in primary-level schools, i.e., pre-primary level school or kindergarten to grade 4 in primary-level schools (Department of Health, 2006). Thus, the aim of this study was to test the effectiveness of iron fortification of milk using NaFeEDTA for the intervention of anemia in school-age children.

Materials and Methods

Sample selection and sample size

All participants were children in primary level schools (Prathomsuksa, grade 4), who could read and write Thai language. Small and medium size schools at Si Chomphu district, Khon Kaen province were random selected according to the proximity to the district center. The other criteria for selection were the teachers' willingness to cooperate and willingness to join the study. All schools were similar in their socioeconomic characteristics. One hundred and sixty students, which consisting of 86 boys and 74 girls out of 1,158 students, voluntary joined the project. They were randomly selected and asked to participate in this study.

Procedures

This study was conducted between November 2007 and March 2008. The purpose of the study was explained to the administrators of all four schools in Si Chomphu district, Khon Kaen province, i.e., Si Chomphu Kindergarten School, Ban Mai Sok Som Kob School, Ban Nong Ta Kai School, and Choom Chon Ban Wang Perm School. The permission to conduct the study was received from local education authorities and school principals. Informed written consent was obtained from the parents or guardians of each child who accompanied the children on the day of the introduction of the survey. De-worming by 15 mg albendazole was provided for all of the students for the elimination of intestinal parasites before starting

the intervention. Based on the study protocol, 5 mg of iron in the form of NaFeEDTA milk (200 ml per sachet) had been administered daily to each subject of the fortified milk group (FM) at 8.30 AM for a period of three months. The intervention group as well as the control group who were given non-fortified milk (NF) was given milk of the same brand and quantity. The quantity of NaFeEDTA was given to the students following the Joint Expert Committee on Food Additives (JECFA) recommendations. Both the fortified and the non-fortified milk were provided by a milk company located within the Khon Kaen province. The concentration of iron added to the fortified milk was 5 mg of iron per 200 ml of milk as NaFeEDTA. Food grade-NaFeEDTA was manufactured and provided by AkzoNobel Chemicals Pte. Ltd., Singapore. The teacher or staff member of the project distributed the milk to each child five days per week. In order to ensure accurate intake, the milk was consumed by the pupils under the complete supervision from the investigator and teachers. At the same time, detailed information about the milk consumption of each child was recorded on a consumption sheet to check the compliance of the students. A dietary assessment was also undertaken to assess macro- and micronutrients intake. The Ethical Committee of Faculty of Medicine, Khon Kaen University, Khon Kaen province, Thailand, approved the study.

Dietary assessment

Three day food record was used to record the number of meals, kind of food and amount of food consumption per day by the portion size estimation. Each student recorded everything that they eat and drink for three days; for two week days and one week=nd under the researcher and dietitian's recommendation, and the data were used to calculate the energy and iron content of food consumed by using the INMUCAL (Institute of

Nutrition, Mahidol University Calculation) program Version 7. According to the Thai's committee of DRI, and adequacy of daily nutrients intake should be at least 67% of Thai DRI.

Laboratory analysis

A 3 ml venous blood sample was taken for the measurement of hemoglobin concentration (Hb), hematocrit (Hct), serum ferritin (SF), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Each sample was drawn into EDTA-coated tubes. The tubes were kept cool and transported to the laboratory at the Research Center, Faculty of Associated Medical Science and Faculty of Medicine, Khon Kaen University. All of bloods samples were analyzed to determine iron storage (serum ferritin) by using COBAS INTEGRA[®] 800 analyzer and red blood cell indices, i.e., Hb, Hct, MCV, MCH, and MCHC, and were analyzed by the Sysmex[®] SF-3000 automated hematology analyzer, Kobe, Japan.

Statistical analysis

The STATA program had been used for statistical calculations. Percent, mean, standard deviation, and median were calculated to describe general information. Students' t-test and Wilcoxon' sign rank test were used to examine the difference in changes of hemoglobin, hematocrit, serum ferritin, mean cell volume, and mean corpuscular hemoglobin concentration over the study period of three months.

RESULTS

It was found that the mean age of the students was 9.9 ± 0.3 and 9.8 ± 0.4 years old for males and females respectively. Mean weight of males and females was 27.7 ± 5.5 kg and 29.7 ± 8.8 kg, respectively, and mean height of males and females was $129.6 \pm 7.8.5$ and 133.4 ± 9.2 cm, respectively.

Dietary surveys

Macronutrients and micronutrients consumption

Overall, the intake of macronutrients was found to be sufficient. Energy consumption was 1,532.8 ± 417.5 kcal vs. 1,528.4 ± 450.1 kcal (92 % vs. 92 % of the Thai DRI for the NF and FM group). The amount of carbohydrate, protein and fat consumed by both groups was also sufficient

compared with the % Thai DRI and results are summarized in Figure 1. There was no statistical difference between groups at P<0.05. As far as micronutrients consumption was concerned, the overall daily intake was inadequate for calcium, elemental iron, vitamin A and vitamin C for both groups except for phosphorus when compared with %Thai DRI.

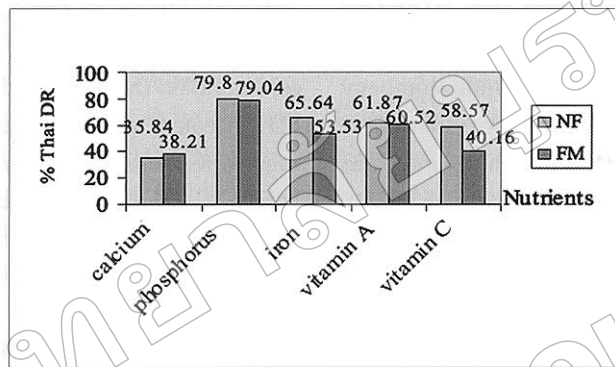


Figure 1. Percent adequacy of micronutrients intake classified by group at baseline.

Hematological assessment

It was found that two male students had Thalassemia by the Dichlorophenol-indophenol (DCIP) screening test for Thalassemia diseases, and

they were excluded from the study. Changes in the hematological parameters and iron status were evaluated and the results are given in Tables 1, 2, and 3.

Table 1. Anemia prevalence and number of changes in prevalence of anemia before and after three months of intervention according to group.

Type of milk	Number of changes in sampling group (sample size, n = 80/group)			Median difference (95% CI)	P-value
	Before intervention	After intervention	Changes in%		
Non-fortified milk	7(8.75)	13(16.25)	7.5		
Fortified milk	15(18.75)	18(22.50)	3.75	0.38 (-0.03 to 0.11)	0.30

The World Health Organization (WHO, 2001)'s criteria (Hemoglobin <11.5g/dL cut off point) were used as criteria to define anemia prevalence. Results in Table 1 show the changes in prevalence of anemia between NF and FM before

and after three months of intervention. A higher prevalence of anemia was still detected after the intervention in both groups, but the difference between both groups did turn out not to be significantly different.

Table 2. Comparison of the red blood cell indices in blood samples taken before and after three months of intervention.

Content of blood sample	Type of milk consumed	Median before study (min, max)	Median after study (min, max)	Median difference (95 % CI)	P-value
Mean cell volume (fl)	Non-fortified milk	76 (54.00, 87.00)	78.80 (55.40, 89.20)	2.05 (1.78 to 2.40)	0.0002*
	Fortified milk	74.00 (54.00, 85.00)	76.40 (56.50, 88.40)	2.60 (2.37 to 2.90)	
Mean corpuscular hemoglobin (pg/cell)	Non-fortified milk	26.00 (18.00, 31.00)	25.90 (17.90, 31.30)	0.15 (0.00 to 0.30)	0.0001*
	Fortified milk	25.00 (18.00, 29.00)	25.25 (18.50, 29.90)	0.50 (0.40 to 0.70)	
Mean corpuscular hemoglobin concentration (g/dl)	Non-fortified milk	34.00 (31.00, 36.00)	33.10 (30.60, 35.00)	-0.80 (-1 to -0.7)	0.0005*
	Fortified milk	34.00 (31.00, 36.00)	33.20 (30.80, 36.50)	-0.60 (-0.70 to -0.30)	

Results in Table 2 depict the changes of red blood cell indices after three months of intervention. The results indicate that intervention statistically altered the difference between before and after the provision of fortified milk of MCV and MCH for the FM group above the NF group ($P < 0.05$). MCHC values for the difference between those two groups did not increase for the intervention when compared to the control group.

Mean values of Hb for the control group decreased while the values of the intervention group increased. The difference between values of before and after the intervention for both groups was statistically significant. The values of Hct in samples taken before and after the intervention were significantly different between the NF and FM group in favor of the latter one. The variation of ferritin was not altered by the intervention, as shown in Table 3.

Table 3. Results of the analysis of the level of hemoglobin, hematocrit, and serum ferritin which were detected in blood samples of children taken before and three months of intervention. Data were analyzed by the student's t- test, and presented as the mean value \pm SD. The sample size (n) was 80 children per group.

Content of blood sample	Type of milk consumed	Before study ($\bar{x} \pm$ SD)	After study ($\bar{x} \pm$ SD)	Mean difference (95 % CI)	P-value
Hemoglobin (g/dl)	Non-fortified milk	12.6 \pm 0.8	12.3 \pm 0.9	-0.3 (-0.4 to -0.2)	0.0004**
	Fortified milk	12.2 \pm 1.1	12.2 \pm 1.0	-0.0 (-0.1 to -0.2)	
Hematocrit (%)	Non-fortified milk	37.45 \pm 2.0	37.3 \pm 2.5	-0.2 (-0.5 to 0.2)	0.01**
	Fortified milk	36.4 \pm 3.0	37.0 \pm 3.2	0.6 (0.1 to 1.0)	
Content of blood sample	Type of milk consumed	Median before study (min, max)	Median after study (min, max)	Mean difference (95 % CI)	P-value
Serum ferritin (ng/ml)*	Non-fortified milk	55.3 (16.5, 72.6)	58.6 (12.4, 186.5)	7.9 (-5.8 to 21.9)	0.08
	Fortified milk	53.2 (20.3, 159.9)	51.3 (14.1, 159.9)	0.7 (-4.2 to 7.8)	

* The data were analyzed by the Mann-Whitney U test and presented as the median value.

** Significant difference at $P < 0.05$.

DISCUSSION

The changes of the hematological indicators prove that iron fortification had a positive effect. It is assumed that the children had been in a slight status of iron depletion but not to such an extent, that ferritin levels could recognize it. However, MCV and MCH can be taken as indirect indicators of the effectiveness of iron fortification on the hemopoietic system, while ferritin and also MCHC failed to respond to iron fortification. It is known that ferritin responds to infection as well and not only to the status of the iron stores (Ash, 2003), and MCHC is known to vary substantially, and in this

study the average values decreased under fortification although the decrease was less pronounced for the intervention group in comparison to the control group. Information about acute infection of the children was not available. Because of ethical reasons it was not possible to conduct the study in groups of children with frank anemia in supporting one group and neglecting somehow the other group. All the children of this study were facing no serious hematological problem and, therefore, the results achieved by iron fortification only can be minimal.

Actually some of the children had been anemic according to WHO standard but only slightly.

Iron fortification in fact was not able to correct that. It might be that a combination of iron depletion and subclinical vitamin A deficiency contribute to the occurrence of anemia and the fact that the anemic status did not disappear under iron fortification (Ash et al., 2003).

The amount of iron in food fortification of other studies usually has been higher than that used in this study (Huong et al., 2006). The use of NaFeEDTA-fortified milk in this study was only 5 mg of iron per person per day which is quite a low dose of NaFeEDTA. So the most plausible explanation for the failure to correct anemic prevalence in this study is the small amount of iron supplemented and the short duration of intervention when compared with other studies (Huong et al., 2005; Andang'o et al., 2007).

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REFERENCES

- Andang'o, P.E., Osendarp, S.J.M., Ayah, R., West, C.E., Mwaniki, D.L., De Wolf, C.A., Kraaijenhagen R., Kok, F.J and Verhoef, H. 2007. Efficacy of iron-fortified whole maize flour on iron status of school children in Kenya: a randomized controlled trial. *Lancet*: May 26; 369(9575): 1799-806.
- Ash, D.M., Tatala, S.R., Frongillo, E.A., Jr., Ndossi, G.D., and Latham, M.C. 2003. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. *American Journal of Clinical Nutrition* 77(4): 891-8.
- Department of Health. 2006. Situation of iron deficiency anemia in school age children. Available at URL: <http://advisor.anamai.moph.go.th/factsheet/student/anemia.htm>. Retrieved on August 25, 2006 (in Thai).
- Food and Nutrition Research Institute (FNRI). 2006. FNRI food and nutrition research institute. Available at URL: <http://www.fao.org/news/1998/981010-e.htm>. Retrieved on February 11, 2006.
- Huong, L.T., Brouwer, I.D., Khan, N.C., Jan, B., and Kok, F.J. 2005. Iron fortification, not deworming improves anaemia of school children in rural Vietnam. *Nutrition Journal* 5: 32.
- Huong, L.T., Brouwer, I.D., Burema, J., Nguyen, K.C., and Kok, F.J. 2006. Efficacy of iron fortification compared to iron supplementation among Vietnamese school children. *Nutrition Journal* 5: 32.
- Institute of Provincial Public Health. 2005. Annual report: Iron deficiency in primary school in Si Chomphu district, Khon Kaen Institute, Khon Kaen province, Thailand (in Thai).
- Seshadri, S. and Gopaldas, T. 1989. Impact of iron supplementation on cognitive functions in preschool and school-age children: the Indian experience. *American Journal of Clinical Nutrition* 50(3 Suppl.): 675-86.
- United Nations Standing Committees on Nutrition (UNSCN). 2006. Nutrition situation and trend. Available at URL: <http://www.org/scn/archives/thailand/ch11.htm#TopOfPage>. Retrieved on August 15, 2006.
- Iron Deficiency Anemia Assessment, Prevention, and Control a Guide for programme managers. Available at URL: http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf. Retrieved on August 6, 2006.