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Research Article

Efficacy of Calcium-Rich Foods Consumption on Urinary Fluoride Excretion in Children: A Pilot Trial in Halaba, Southern Ethiopia

Tamirat Getachew Bantero[1], Demmelash Mulualem[1], Derese Tamiru Desta[1], Getahun Wansisa Worancha[2], Bergene Boshe Boricha[1]* and Susan J.Whiting[3]

[1]School of Nutrition, Food Science and Technology, Hawassa University, Ethiopia
[2]Faculty of Biosystems and Water Resources Engineering, Institute of Technology, Hawassa University, Ethiopia
[3]College of Pharmacy and Nutrition, University of Saskatchewan, Canada

***Corresponding author**

Bergene Boshe Boricha, School of Nutrition, Food Science and Technology, Academic Center of Excellence for Human Nutrition, Hawassa University, P.O.BOX 05, Hawassa, Ethiopia, Tel: 251916127061/+251954727909

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Abstract

Fluorosis is a public health problem in the Rift Valley areas of Ethiopia. While defluoridation of water is best solution, consumption of calcium-rich foods may mitigate development of adverse symptoms. The aim of the study was to determine whether calcium-rich foods would decrease fluoride absorption in children using urinary fluoride concentration as an indirect measure. Randomly selected children from a larger study were assigned to four groups of 10 participants each: two groups had a daily calcium-rich food as the intervention, one as a solid food (millet porridge) and one as a beverage (cup of milk); and two groups acted as calcium-poor controls also using a solid food (maize porridge) or a beverage (cup of soft drink). Morning urine was collected at baseline and at end line after 7 days. After the seven days, mean (SD) urinary fluoride concentration in the combined calcium-rich intervention groups was significantly (p=0.006) reduced from 6.8 (2.5) to 4.1 (2.4) mg/L. No significant (p>0.05) change was seen for the combined calcium-poor controls. Children in areas having high fluoride water would benefit from calcium-rich foods to prevent fluoride absorption causing dental and skeletal fluorosis.

ABBREVIATIONS

F: Fluoride; Ca: Calcium

INTRODUCTION

Fluoride (F) occurs in high concentrations in ground water in many parts of the world [1]. Topically applied F has a protective effect against dental caries [2] and as such is an essential micromineral with an Adequate Intake (AI) recommended for children ages 4-8 years of 1 mg/day [3]. However, F in doses over 0.1 mg F/kg body weight/day can weaken the skeleton and teeth as well as cause non-skeletal problems such as gastrointestinal upset, and pain [4,5]. The Upper-Tolerable Intake Level (UL) is set to prevent excess intake, and the UL value for children ages 4-8 y is 2.2 mg/day, showing a very narrow safe range of intake between AI and UL [6]. In areas where drinking water with F concentrations exceed the World Health Organization (WHO) guideline of a "desirable" upper limit of 1.5 mg/L [7], severe health effects of dental fluorosis (DF) and skeletal fluorosis (SF) are seen in those populations [4, 8, 9]. In the Ethiopian Rift Valley, the mean level of fluoride in ground water is 6.03 mg/L and which is 4-fold higher than the WHO upper limit, indicating high risk for fluorosis by those who depend on this water source [10].

much as 80% of ingested fluoride from dietary sources (water, prepared food and beverages) is absorbed in the stomach [3]. Plasma distributes F to hard tissues, and plasma F levels reflect what has been absorbed. Urinary excretion of F rises when F intake is high and is an indirect indictor of F absorption [8, 11,12]. When ingested F absorption is limited by the co-ingestion of divalent cations such as calcium and magnesium, urinary F levels do not increase, indicating that F ingestion has been blocked. However, there are only a limited number of studies in animal models [12], and adults [13], demonstrating this.

Recently, a study of school-age children in Southern Ethiopia showed that they had low calcium intakes, and that greater severity of dental fluorosis was associated with this low calcium intake in an area where water F was over 10 mg/L [5]. This study revealed that fluorosis remains a significant community health problem in Ethiopia and that there is excess intake of fluoride and low consumption of dietary calcium in Halaba. In adults, a 6-month study was conducted [13], whereby supplemental intake of 1000 mg calcium per day reduced urinary F excretion in women by approximately 60%.

As children are prone to dental fluorosis, it is important to determine whether providing additional calcium to children could impair F absorption and thus be a possible mitigating factor in preventing fluorosis. To our knowledge only one study in older children has measured a fall in urinary F concentration after increasing dietary calcium intake [14]. We aimed to determine whether urinary F excretion would be reduced using food sources of calcium in young children. We tested two common sources of calcium food for children in Ethiopia: milk and millet, and paired each with a control food that was similar in energy intake and food type, i.e., soft drink and maize, respectively. Our hypothesis was that those children given the calcium rich food would have a lower urinary F excretion than control children who received the low-calcium rich food.

MATERIALS AND METHODS

Study area

Halaba zone is located 315 km south of Addis Ababa and is geographically located at 7017’ N latitude and 38006’ E longitude. The altitude of the study area ranges from 1554 to 2149 m with most of the area being about 1800 m. Rainfall has been a major limiting factor, thus creating a reliance on ground water as a source of water. The study area was selected because it was known to have high water fluoride [11]. Two kebeles (Kobochobare and Andegna Ansha) were chosen for a larger study of the area, as previously described [15]. In brief, a list of households with children 3-8 years were identified, and 127 study participants (parent-child pairs) were selected using simple random method from each kebele. From this larger group, a total of four groups of 10 children each were randomly selected from Kobochobare kebele (Figure 1). In households where there was more than one eligible child, the index child was selected by tossing a random number. The study was conducted from November to December, 2016.

Fluoride and calcium intakes

At the time of the larger study, water and staple foods were selected from each area to determine fluoride intakes as well as calcium sources in the community. Water samples from the source, urine sample and staple foods were collected from the study participants, households, and community shopping centers. The collected samples were kept in polyethylene bags on ice until transported to laboratory [15].

Intervention, measurement, and data collection procedures

The 40 children were selected from Kobochobare kebele by the random selection and assigned randomly into two arms of the study (Figure 1). Baseline urine samples were collected before the feeding trial began.

In the first arm, one group was given a daily intake of 250 mL of milk (calcium-rich) and the other group was given a daily intake of 250 mL of soft drink (calcium-poor). In the second arm of the study one group was given 100 grams of millet porridge (calcium-rich) and the other group consumed 100 grams maize porridge (calcium-poor). Beverages and food ingredients were purchased locally and distributed daily, by the researchers, at the nearest health post. Before cooking porridges, millet and maize grains were washed, dried and milled. To make 100 g millet porridge, the following ingredients were mixed and cooked: 35 g of millet flour, 123 g of water, 0.54 g of iodized salt and 5.4 g of vegetable oil. To make 100 g maize porridge, the following

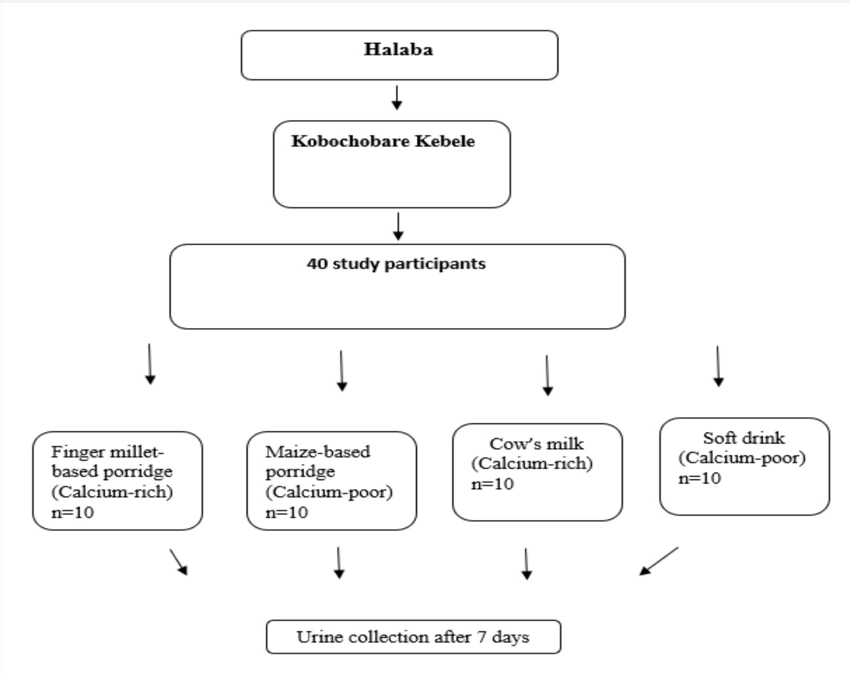

Figure 1: Flowchart showing sampling and treatments.

ingredients were mixed and cooked: 31 g of maize flour, 108 g of water, 0.5 g of iodized salt, and 4.7 g of vegetable oil. All the ingredients used for the preparation of porridges were purchased from local market of the kebele, and cooking was carried out at the health post.

Intervention

Each child was given 100 g of porridge at the health post, which they ate completely. For beverages, exact amounts of milk or soft drink were provided to each child. Leftover foods from cooked porridge or beverages were given to the family for other children. The study participants' caregivers were instructed to have children eat their usual amounts of food, beverages, and water in addition to the provided food or beverage provided.

Foods were provided for seven days. In the morning of the eighth day, the child's first-morning urine was collected in a coded plastic tube at the health post. Urine samples were quickly stored in icebox cooled with ice packs until brought to the Academic Center of Excellence for Human Nutrition laboratory of School of Nutrition, Food Sciences and Technology at Hawassa University.

Laboratory analysis

The fluoride levels of urine were analyzed at the Ethiopian Public Health Institute (EPHI) laboratory, by using an ion analyzer Orion Research Specific Ion meter, as described elsewhere [15]. Then urinary fluoride, food, and water samples were analyzed by using Perfect IONTM combination fluoride ion-selective electrode (Mettler Toledo, Germany) coupled with bench top dual-channel ion-meter (Jenway, model 3345, England) and pH/ISE meter (Orion Model, EA 940 Expandable Ion Analyzer) equipped with combination fluoride-ion selective electrode (Orion Model 96-09). The calcium contents of water and food samples were determined by using atomic absorption spectrophotometer by using the AOAC method (2000). The staple food and water sample F levels were analyzed, and the result described in mg/kg or ml/L.

The amounts of calcium and fluoride were calculated manually using two stages [16]. In the first stage, the nutrient intake data were recorded separately for each respondent. Portion sizes and quantities were converted into weight equivalents. In the second stage, for each respondent, the weight equivalent was computed manually using the Ethiopian Food Composition table for calcium and laboratory analysis results for fluoride.

Data management and analysis

All data were entered, cleaned, and analyzed using statistical package for social science software (SPSS) [17]. Using fluoride and calcium values for foods and beverages, mean intake, percent of recommended daily allowance, and upper tolerable intake, and descriptive statistics (mean ±SD), frequency and percentage) were calculated as previously described [15]. The trial data was calculated for comparison of mean and significant difference between two treatment groups and controls using independent two samples t-test. P-value of less than 0.05 was taken as statistically significant.

Ethical considerations

The study approval was obtained from the Institutional Review Board of the College of medicine and health science at Hawassa University. Informed written consent was taken from the study participants' mothers.

RESULTS AND DISCUSSION

The study subjects in this study consisted of 40 children, 22 were males and 18 were females. The mean age of children was 4.32 ± 0.92 years, ranging in from 3 to 6 years. Details of these children were provided previously [15]. The intervention provided approximately 140 mg additional calcium per day when children were fed millet porridge compared to those given maize porridge, and approximately 200 mg additional calcium when the control children were provided with cow's milk as opposed to soft drink (Table 1).

In Table 1, urinary fluoride concentrations are shown for all four groups. The concentration of fluoride changed from 6.1±2.5 to 3.8±2.6, 6.5±2.3 to 7.5±4.6, 7.6±2.3 to 4.5±2.3 and 4.9±2.1 to 5.0±1.9 for millet, maize, cow milk group and soft drink groups, respectively, from baseline to endline. There was an apparent decrease in the calcium-rich groups (with that for milk being significant) while values in the calcium-poor groups did not change. In Table 2, data from the results of combining data for feeding calcium-rich foods versus calcium-poor food are shown. When children were given the calcium-rich treatments for 7 days, there was a significant decrease in urinary fluoride excretion at endline compared to baseline. In contrast, when children were given a calcium-poor food or beverage, no change in fluoride excretion was observed.

It was found that urinary F excretion was significantly reduced when children were given a calcium-rich food (millet porridge) or beverage (milk) every day for 7 days. In contrast,

Table 1: Mean (±SD) urinary fluoride level of children assigned in different groups during the seven days intervention period in Halaba, Ethiopia

Groups	Extra Calcium[#]	Urinary Fluoride (mg/L)	
		Mean (SD)	
N = 10 per group	mg/day	Baseline	Endline
Calcium-rich food Millet porridge 100 g	142.5	6.1 (2.5)	3.8(2.6)
Calcium-poor food Maize porridge 100 g	2.3	6.5 (2.3)	7.5 (4.6)
Calcium-rich beverage Cow milk 250 mL	222.5	7.6 (2.3)	4.5 (2.3)
Calcium-poor beverage Soft drink 250 mL	5	4.9 (2.1)	5.0 (1.9)

[#] Estimation of calcium content was made using Ethiopian Food Composition and United Stated Department of Agriculture Food Composition Tables.

Table 2: Independent Sample t-test of urinary fluoride level of among children who consumed calcium-rich and calcium-poor foods during the 7 days intervention period in Halaba, Ethiopia

Period	Calcium-rich foods (n =20)	Calcium-poor foods (n=20)	*t* (df)	*P*	95% CI
Baseline	6.8 (2.5)	5.7 (2.3)	1.52 (38)	0.142	-0.40, 2.68
Endline	4.1 (2.4)	6.9 (3.5)	-2.95 (38)	0.006[#]	-4.75, -0.89

[#]Statistically significant (P<0.05) difference observed using Independent Sample t-test
Abbreviations: CI; confidence interval; df: degrees of freedom. P: probability

in children given the calcium-poor foods maize porridge or soft drink as a control food, urinary F excretion remained the same. Daily estimated amount of calcium intake without the intervention foods was approximately 360 mg, as we previously reported [15], while adding a serving of a calcium-rich food increased intake to an average of 500 – 580 mg/day. This would result in more children meeting their RDA of 1000 mg per day for children 4 to 8 years [18]. A study of older children in Halaba, age 6 to 8 years of age, found dietary calcium to be 544 ± 203 mg [5]. This is comparable as older children have higher energy demands and consume more food as a result.

At baseline urinary F excretion for the four groups averaged 6.2mg/L per day, which was indicative of the high level of fluoride these children were exposed to. After seven days of feeding the intervention or control foods, urinary fluoride excretion decreased in the children given the calcium-rich foods (millet porridge or cow's milk) and rose in children given the calcium-poor foods (maize porridge or soft drink). When data were analyzed by combining both calcium-rich groups and calcium-poor groups (Table 2). So that the number of subjects was 20 in each group, thus increasing power, there was a significant decrease in urinary F excretion with calcium-rich foods compared to no change with calcium-poor foods.

The current study showed that children living in a high fluoride water environment had a high excretion of urinary fluoride. Similar findings were seen in Ethiopian children living in Halaba by [11], who showed 24-hour takes of F being 12.9 ± 3.8 mg, which would result in similarly high excretion rate of F. Halaba is not the only region in Ethiopia with high F intakes. As shown by Demelash et al. [10], throughout the Ethiopian Rift Valley, the pooled mean level of fluoride in ground water was 6.03 mg/L. Kebede et al. [11], had also reported high F intakes in several sites in Ethiopia.

We found that when examined as pooled intake, those children given the calcium-rich foods, either millet porridge or cow's milk, had significantly less urinary F than children given the low calcium-poor food of maize porridge or soft drink, respectively. Previous studies support that fluorosis can be mitigated by increasing calcium intake by a therapeutic regimen including calcium [19]. We have shown in adults living in Ethiopia a calcium supplement (which was eggshell powder) decreased urinary fluoride excretion and reduced signs and symptoms of fluorosis [13]. A study in children showed that a low dietary calcium intake was associated with greater risk of dental fluorosis and signs of skeletal fluorosis [5]. Our current study has shown that even over a short time period (seven days consumption) of calcium-rich food, there can be a reduction in the concentration of urinary fluoride which is an indirect measure of a reduction in fluoride absorption [8].

The mechanism for calcium's mitigation of F absorption is thought to be binding of the F- anion with the Ca++ cation producing an insoluble compound that cannot be absorbed [20]. In an animal study, excretion of fecal F increased and urinary F decreased when rats were fed high F diets, which supports this mechanism [12].

Calcium is not the only nutritional supplement that protects against fluoride absorption. In a study done on 30 adolescent dental patients, one group received 250 mg calcium with a vitamin D3 supplement compared to 500 mg ascorbic acid also with vitamin D, for three months. Similar to our results, urinary F excretion was reduced with calcium, and its effect was greater than that of vitamin C [21]. However, in our study, the foods provided were locally available and acceptable, in contrast to supplemental nutrients such as vitamin C.

Calcium intakes in Ethiopia are low, as shown by [22], for children and by [23], for adult women. In our larger study [15], we found that the community had low level of understanding on the prevention of fluorosis and were not aware of the benefit of calcium rich foods on the fluorosis prevention. Like as many of Ethiopian community, this community uses cereals like maize, and other cereals and root and tubers for complementary food preparation and for household consumption. These foods contain low amount of calcium. Therefore, providing nutrition education, accessing foods which contain good amount of calcium like milk and finger millet is important to prevent the problem in the sustainable manner. As recently shown [24], finger millet is one of the most calcium-dense foods, with three times the level of calcium than milk, and the only cereal that contains high calcium content which is consistent across different varieties. Thus, it and other high calcium foods that are locally available should be promoted in high fluoride areas of Ethiopia [15].

The limitations of this study include that the effect of calcium on body fluoride was measured using urinary fluoride excretion which is an indirect measure of F absorption. Further, dietary calcium intake of children was estimated from a week-long frequencies of calcium-rich food consumption which might not have accurately estimated the usual calcium intake of children beyond that week. Finally, the randomization was done without blinding. Nevertheless, our study provide support for improving calcium intakes of young children, especially in areas where water fluoride levels are high.

CONCLUSION

Dietary intakes of calcium were low in this area of Ethiopia which experienced high levels of fluoride in water and foods.

Subsequently the children were at risk for dental and skeletal fluorosis in previous reports from this area. In testing whether a calcium-rich food added daily would reduce the burden of excess fluoride absorption leading to fluorosis, we found one cup of milk (approximately 250 mL) reduced urinary F excretion which indirectly demonstrated reduced fluoride absorption. In general adding calcium-rich foods such as millet may also be as effective.

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