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ORIGINAL ARTICLE

Safety evaluation on pH and fluoride content of locally available and packaged milk as a potential factor in dental fluorosis in Jaipur, India

Preeti Vishnani, Anup Nagaraj, Asif Yousuf[1], Shravani Ganta, Sonia Pareek[2], Siddharth Acharya, Kushpal Singh[3]

Department of Public Health Dentistry, Jaipur Dental College, [2]Department of Public Health Dentistry, Government Dental College, Jaipur, Rajasthan, [1]Department of Public Health Dentistry, Government Dental College, Srinagar, Jammu and Kashmir, [3]Department of Public Health Dentistry, Panineeya Institute of Dental Sciences & Research Centre, Dilsukhnagar, Hyderabad, India

Address for correspondence:
Dr. Asif Yousuf,
Department of Public Health Dentistry, Government Dental College, Shireen Bagh, Srinagar - 190 010, Jammu and Kashmir, India. E-mail: asify11@gmail.com

ABSTRACT

Objective: Milk being the most consumed food in the 1st years of life and the fluoride exposure through infant foods including milk, during the critical period of tooth development may lead to dental fluorosis. Thus, the aim of the study was to assess and compare the pH using digital pH meter and fluoride content using the spectrophotometric method of locally available and packaged milk in Jaipur. **Materials and Methods**: Fluoride and pH analysis were carried out among 3 milk groups: (i) Locally available milk (ii) packet milk and (iii) packaged tetra-pack milk. A total of 19 samples were included in the study. **Results**: The mean fluoride content was found to be 0.017 (±0.003 standard error of mean) with a range of 0.001–0.02. The pH of the milk samples was found to be in the range of 4.46–7.11. **Conclusion**: In view of the results of the present study, milk consumption has no major impact on total daily dietary fluoride intake, but the pH of the soy-based tetra-pack milk was found to be less than critical pH.

Key words
Dental fluorosis, fluoride, milk

INTRODUCTION

The fluoridation of milk is an attempt to provide benefits of fluoride without requiring the consumers to take on particular responsibilities or change their behavior. The potential of milk as an alternative vehicle for fluoride was first reported from Switzerland by Zeigler in 1962.[1,2] Fluoride is an essential nutrient and has been recognized to be physiologically essential for the normal growth and development of human beings. A level of 0.05–0.07 mg/kg body weight is often thought of as "optimal," however higher levels of intake have been associated with fluorosis. The optimal level is virtually impossible to calculate because of variations in fluoride levels in all sorts of foods and beverages.[3]

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The deciduous teeth undergo substantial mineralization during the 1st year of life, and fluoride provided during this period is reported to give significant protection against caries in deciduous teeth, when available from birth onward. During the 1st year of life, besides rapid bone growth, enamel formation in the primary teeth is being completed, and hard tissue formation has begun for the permanent central, lateral, canine, and first molar teeth. When systemic fluoride is incorporated into the developing teeth, it is believed to increase the crystallinity of the enamel and possibly also to enhance the morphogenesis of the teeth by making the pits and fissures shallower. Therefore, fluoride has been widely used as a caries preventive agent both topically and systemically.[4]

Fluorosis is a toxic manifestation of chronic (low-dose, long-term) fluoride intake. To prevent fluorosis from occurring in the most prominent and or most susceptible teeth, the most critical time to avoid fluoride exposure is the first 3–6 years of a child's life.[5] In India, the fluoride levels in groundwater vary substantially in different regions. High concentrations of fluoride (>1.5 mg/L) have been reported in some of the areas of states of Haryana, Delhi, Rajasthan, Karnataka, and others.[6] In 23 districts of Rajasthan state, the fluorosis problem can be visualized at various intensity levels, that is, dental fluorosis, nonskeletal manifestations and skeletal fluorosis manifesting in the form of chronic joint pain, arthritic symptoms, and increased osteosclerosis. It was reported by Rajasthan Voluntary Health Association in 1994 has showed that the total number of villages having fluoride problem in Rajasthan is 2433 covering nearly 2.6 million population. Moreover, nearly 30,000 people are drinking water with a concentration of 10.0 mg/L of fluoride.[7]

One of the suggested causes of enamel fluorosis is an increase in dietary fluoride intake by children.[8] The amount of fluoride consumed during infancy and early childhood has been reported to be closely related to the consumption of powdered milk and the fluoride concentration in the local drinking water.[9] Milk is universal food for infants and children.[10] It provides energy and essential nutrients-proteins, fats, carbohydrates, vitamins, and minerals.[11] Milk can be fortified with Vitamins A and D.[12] Being a high fluoridated belt area, nurtured a hypothesis whether fluoride intake through milk has any impact on dental fluorosis. While small amounts of fluoride in food have been shown to help prevent tooth decay, too much fluoride in the diet or long-term excessive intake of fluoride can result in macular teeth.[10] Literature review[13,14] reveals that the fluoride content of cow's milk and milk formulas were in the range of 0.02–0.8 ppm. Liu *et al.*[4] reported that, the fluoride content of milk samples available from supermarkets was low from 0.007 to 0.068 ppm. Milk contains 4–5% disaccharide lactose, which can be fermented by oral biofilm bacteria. Normally, sucrose lowers the plaque pH to below 5.0, whereas lactose lowers it to around 6.0. The carbohydrate content of milk confers a low cariogenic potential.[15,16] Birkhed *et al.*[17] found that milk induced minimal pH reduction compared to fruit juice and sweetened beverages. Despite the fact that, milk and milk products are consumed commonly, there is remarkably little clinical research concerning their effect on oral health and diseases.[16]

It is the purpose of our study, to assess and compare the pH and fluoride content of locally available cattle milk and packaged milk commonly consumed in Jaipur, Rajasthan, India.

MATERIALS AND METHODS

The present study was a cross-sectional *in-vitro* study to evaluate the pH and amount of fluoride in the locally available and commercially available milk in Jaipur city of Rajasthan. The research protocol of the study was reviewed, and ethical clearance was obtained from Institutional Review Board of Jaipur Dental College. A total of 19 milk samples were collected and analyzed for fluoride content and pH values. These 19 samples were categorized into two groups: Locally available - 4 milk samples and commercially available 15 samples. The commercially available samples were again divided into two groups - Packet milk (8 samples) and tetra-pack milk (7 samples). Among the 7 samples of tetra-pack milk, one was soy-based milk. Different brands of commercially available milk were purchased, from the market on two different days so that the batch number was different for each sample. Two different samples of locally available milk were also collected from the local dairy farm. All the samples were transferred to similar containers and then, coded by an assistant to ensure that the investigator, laboratory technician and statistician were all blinded.

Fluoride was analyzed using spectrophotometric method (UV-VIS Spectrophotometer 119, Systronics). About 5.0 cm^3 each of alizarin red and zirconyl acid solutions were added to 100 cm^3 of both standard and sample solutions mixed thoroughly and allowed to stand for 1 h for full-color development. Absorbance readings were taken at 520 nm. A calibration curve was prepared from the plot of absorbance against the concentration of standard solutions. The concentrations of the sample solutions were determined from the plot. Where necessary values obtained were multiplied by an appropriate dilution factors to get actual concentrations.[18,19]

The pH of the milk samples were measured using the digital pH meter. All the samples were analyzed in duplicate. Qualitative data thus collected were summarized as mean and standard deviation (mean ± standard error of mean [SEM]). The mean levels of fluoride content between locally available and packaged milk were compared using ANOVA. The significance level was set at $P < 0.05$. All analysis was performed using MedCalc (v12.2.1.0, MedCalc Software, Mariakerke, Belgium).

RESULTS

The fluoride was determined for different milk samples in parts per million (ppm). Two samples of milk per variety were analyzed, and the mean was calculated. The mean Fluoride content was found to be 0.017 (±0.003 SEM) with a range of 0.001 ± 0.0001–0.02 ± 0.0001 [Table 1]. The pH of milk samples was found to be in the range of 6.2500 ± 0.0850–7.0000 ± 0.1100, but the pH of soy-based milk was found to be 4.3775 ± 0.0175 [Table 2].

DISCUSSION

Milk is the most consumed food in the 1st years of life. Various studies have validated that it is obligatory to know the fluoride concentration of infant foods, foodstuffs and beverages to estimate the total amount of fluoride ingestion by children. Although, it is difficult to determine precisely the total fluorine intake from the diet, it is certain that there is considerable variation in the intake

Table 1: Fluoride content of locally available and packaged milk

Milk brands	Mean±SEM	Range	ANOVA		Significantly different from
			F ratio	*P*	
Locally available					
A	0.0031±0.0002	0.002965-0.00333	25.629	<0.001	4, 11, 12, 17
B	0.0059±0.0002	0.00569-0.006105			4, 11, 17
C	0.0089±0.0001	0.00873-0.00897			11, 13, 14, 17
D	0.0137±0.0045	0.009165-0.01825			All except 3, 4, 5, 12
Packet milk					
PM-A	0.0073±0.0002	0.00718-0.007475			11, 17
PM-B	0.0047±0.0002	0.004485-0.004845			4, 11, 17
PM-C	0.0035±0.0000	0.00351-0.00356			4, 11, 12, 17
PM-D	0.0030±0.0001	0.002905-0.003055			4, 11, 12, 17
PM-E	0.0046±0.0001	0.00451-0.004635			4, 11, 17
PM-F	0.0022±0.0001	0.00209-0.00221			4, 11, 12, 17
PM-G	0.0211±0.0021	0.01902-0.023255			All
PM-H	0.0115±0.0006	0.010935-0.0121			1, 7, 8, 10, 11, 13, 14, 16, 17, 18, 19
Tetra-pack milk					
TPM-A	0.0010±0.0001	0.00081-0.001095			3, 4, 11, 19
TPM-B	0.0013±0.0001	0.00123-0.00135			4, 11, 19
TPM-C	0.0067±0.0003	0.00636-0.00697			4, 11, 19
TPM-D	0.0022±0.0002	0.001985-0.002335			4, 11, 19
TPM-E	0.0223±0.0009	0.021385-0.02322			All except 11
TPM-F	0.0044±0.0000	0.004405-0.0044450.003205-0.00582			4, 11, 19
TPM-G	0.0045±0.0013	0.003205-0.00582			4, 11, 19

SEM: Standard error of mean

Table 2: pH content of locally available and commercially available milk

Milk brands	Mean±SEM	Range	ANOVA		Significantly different from
			F ratio	*P*	
Locally available					
A	6.7875±0.0675	6.72-6.855	48.563	<0.001	4, 15, 17, 18
B	6.7075±0.0025	6.705-6.71			18
C	6.3575±0.0225	6.335-6.38			6, 11, 12, 18
D	6.3050±0.0100	6.295-6.315			1, 6, 11, 12, 18
Packet milk					
PM-A	6.6775±0.0325	6.645-6.71			18
PM-B	7.0000±0.1100	6.89-7.11			3, 4, 13-19
PM-C	6.7025±0.0425	6.66-6.745			19
PM-D	6.7450±0.0650	6.68-6.81			17, 18
PM-E	6.6850±0.0200	6.665-6.705			18
PM-F	6.6550±0.0050	6.65-6.66			18
PM-G	6.9250±0.1300	6.795-7.055			3, 4, 14 to 18
PM-H	6.9550±0.1250	6.83-7.08			3, 4, 14 to 18
Tetra-pack milk					
TPM-A	6.5100±0.0300	6.48-6.54			6, 18
TPM-B	6.3800±0.0800	6.3-6.46			6, 11, 12, 18, 19
TPM-C	6.3175±0.1725	6.145-6.49			1, 6, 11, 12, 18
TPM-D	6.3675±0.1375	6.23-6.505			6, 11, 12, 18
TPM-E	6.2500±0.0850	6.165-6.335			1, 6, 8, 11, 12, 18
TPM-F	6.4675±0.0425	6.425-6.51			6, 12, 18
TPM-G	4.3775±0.0175	4.36-4.395			All

SEM: Standard error of mean

and fluoride content of different foods, canned juices, carbonated beverages, infant formulas, commercially prepared infant food. Since products are not required to have their fluoride content displayed, only a fluoride assay is possible to determine the dietary fluoride intake.[4,14,20-25] Rajasthan being a fluoride belt area, the fluoride content in water is high and the fluoride intake of the cattle and inhabitants of the region is beyond the stipulated limits. This in turn may have an impact on the fluoride content of raw milk and even the processed milk. According to a study conducted by Nagaraj *et al.*[12] only half of the children in Jaipur are exclusively breastfed, which indicates more use of cattle milk and processed milk by the people. Hitherto, in the present study a total of 19 samples were analyzed for fluoride concentration, which included both raw and processed milk.

Based on the literature appraisal, the optimal level of fluoride intake was considered to be in the range of 0.05–0.07 mg F/kg body weight,[26] the analyzed milk samples could not contribute significantly to the total ingestion of fluoride, as the maximum level observed was 0.02 ppm. The fluoride content of processed milk may be influenced by the interaction between fluoride and milk, as well as by the wide variety of heat-treatment processes used for pasteurization.[26-28] A wide variation in the fluoride content of locally available milk and packaged milk has been observed in the current study. The variation in fluoride content between different brands and types of milk may be due to differences in the source of "raw" milk, and the fluoride level in water of factories where milk was being processed.[4]

To commensurate with the present study, the fluoride content of milk available in Jaipur was found to be in the range of 0.001–0.02 ppm (mean - 0.017 ppm) which is not kindred to the values of fluoride level of cow's milk reported by Tinanoff and Mueller,[13] Vlachou *et al.*[14] and Latifah and Razak.[29] This discrepancy is also seen when compared with the findings outlined by Silva and Reynolds[30] that the average fluoride content in the infant formulae available in Australia was 0.240 ppm. Our findings are also not in agreement with a recent report by Nohno *et al.*[31] As per a previous report by Liu *et al.*,[4] the present study also demonstrates a large variation in the fluoride content of different brands of milk and even in the same brand. Whereas, Pagliari *et al.*[26] and Lodi *et al.*[20] divulged that there is a significant difference in fluoride content of different batch numbers, which is antithetical with the present study as there was no variation in fluoride concentrations among the different batch numbers of same brand. Johnson and Bawden[32] and Liu *et al.*[4] proclaimed that soy-based infant formulas contain more fluoride than milk-based formulas, whereas in the present study, it was discerned that there was no statistically significant difference among the fluoride content of soy-based milk and natural milk. Furthermore, Mcknight-Hanes *et al.*[33] have revealed that soy-based ready to feed infant formula contain 0.30 mg/L fluoride, which was much higher than the fluoride values of soy-based milk in our study. However, fascinatingly, the pH of the soy-based milk was significantly lower than the other types. This outcome is divergent with the observations of Park[34] and Lutchman *et al.*,[35] in which they unveiled that goat milk had the highest buffering capacity. In conformity with the findings of a study conducted by Opydo-Szymaczek and Opydo,[36] the concentration of fluoride in infant and toddler formulas was low, and the formula itself was not a significant source of fluoride exposure.

Various studies corroborated that fluoride present in the water was the major contributing factor for both systemic and dental fluorosis. To some extent, fluoride taken through other sources like dietary fluoride might also be a contributing factor.[37-39] Fluorine exposure through infant food during the critical period of tooth development may lead to fluorosis. Enamel fluorosis can occur following either an acute or chronic exposure to fluorine during tooth formation. Fluorosed enamel is characterized by retention of amelogenins in the early maturation stage of development and by the formation of more porous enamel with subsurface hypomineralization.[40]

Milk is considered a suitable vehicle for substances beneficial to oral health, principally fluoride.[11] Milk, with a pH ranging from 6.4 to 6.7, is considered to be a functional food that fights cavities because it promotes tooth remineralization and inhibits the growth of plaque.[41] Bibby *et al.*[42] found that the inclusion of milk solids reduced the cariogenicity of sugar-containing foods. Jenkins and Ferguson[43] suggested that the negligible fall in plaque pH was partly due to milk's high buffering power, and the low level of dissolution of test enamel was due to the protective action of milk's high levels of calcium and phosphate.

The low fluoride content of all of the types of milk tested insinuates that milk is not a major contributor to total dietary fluoride intake. Since health professionals use the fluoride content information to evaluate at risk populations for excessive fluoride consumption, continued monitoring, and evaluation of foods and beverages by FCI is needed to provide current fluoride content data. Results from the present investigation show that the milk samples were collected only from one part of Jaipur. The samples should be collected and analyzed for their fluoride content covering a wider area.

Milk consumption has no major impact on total daily dietary fluoride intake, but the pH of the soy-based tetra-pack milk was found to be less than critical pH. Infants and children who are allergic to cow's milk are often substituted. Therefore, the providers need to take into consideration the pH values of the milk that is being consumed. The government should restrict the distribution of commodities such as milk and water from endemic fluoride area to nonendemic fluoride area to overcome the "halo effect." It should be noted that these conclusions are based on the total fluoride content and pH of analyzed products.

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