Changes in plaque fluoride levels by school-based fluoride rinsing and tablet programs in Bangladesh

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The aim of this study was to evaluate the effects of school-based fluoride rinsing and tablet programs on plaque fluoride levels. A total of 42 children (8–9 years) were selected from two neighboring schools in Dhaka, Bangladesh. After caries recordings, vestibular plaque samples from the maxillary central incisors and mandibular first molars were collected and frozen on day 0 (baseline). The subjects of one school (rinsing group, $n = 15$) rinsed with 0.05% NaF, while those from the other school (tablet group, $n = 16$) used a 0.5 mg F tablet on the 5 school days during a 3-week period. The surfaces were sampled on the first and last school day every week. The fluoride and protein contents of each sample were analyzed using micro-techniques. The median plaque fluoride levels were 9.1 ppm at baseline in the rinsing group and 2.5 ppm in the tablet group ($P < 0.05$). This difference could in part be related to reported fluoride toothpaste usage. After 4 days on the fluoride programs, plaque fluoride levels in the rinsing group increased to 27.3, 24.5 and 14.2 ppm in the 3 consecutive weeks. The corresponding values after tablet usage were 8.0, 6.5 and 7.1 ppm, respectively. After 3 days without fluoride during the weekends, levels declined towards baseline values in both groups. Hence, the plaque fluoride levels in 8 to 9-year-old Bangladeshi children were increased by both rinsing and tablet programs, but the effect was not detectable 3 days later. □ *Caries prevention; dental plaque; fluoride rinses; fluoride tablets; plaque fluoride*

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The caries prevalence in Bangladesh has been reported to be low (1, 2). However, children with serious caries problems were quite often seen in an emergency dental care program operating in a slum area in Dhaka, Bangladesh (according to personal communication). Owing to ongoing changes in diet and life style, increasing caries prevalences have been predicted in this country (3). The fluoride (F) concentration in the drinking water has been reported to be low and F toothpaste is not widely used (1). It was therefore decided to test alternative F measures in this slum area as a basis for future preventive programs.

Total plaque F increases after topical F treatments (4–6), and plaque F uptake has been used as a parameter to assess the caries preventive effect of such programs (7). However, most studies on plaque F refer to plaque pooled from several tooth surfaces or from the whole dentition and include a limited number of subjects. The aim of this study was to monitor the changes in plaque F levels at specific tooth sites after daily use of F rinses or tablets.

Materials and methods

The research protocol was approved by the Director of both Primary Health (School Health) and Education in Bangladesh.

Study fields and drinking water F

The study was carried out in two neighboring primary schools in the Dhaka metropolitan area in Bangladesh. One school, Zigatola Government Primary School (Z), is situated adjacent to a slum area. The other, Mohammadpur Government Primary School (M), is situated closer to the city center. Both schools receive water piped from the same source. Water samples from each school, analyzed in low-level TISAB buffer with a combined F-electrode, were all found to contain 0.02 ppm F.

Test subjects

In the M-school, the students from one class of grade III ($n = 20$) were selected for the 'rinsing group'. The subjects ($n = 22$) for the 'tablet group' were chosen from the same grade of the Z-school. All were 8–9 years of age. In both classes, there was a predominance of male students. Parents were informed about the programs and gave their consent. In addition, they were requested to provide information about family income and the use of F toothpaste by their children. All participants were asked not to use F toothpaste during the study and to refrain from toothbrushing 36 h before sampling. Four out of five children in the tablet group had parents with income

below the national average for urban areas (8), whereas this relationship was reversed in the rinsing group.

Caries prevalence

The recordings were made according to WHO criteria (9) by the 1st author, who had been calibrated with the 2nd and 3rd authors. The prevalence in the permanent dentition was low in both groups (mean DMFS 0.1), whereas that in the primary teeth was higher (mean dmfs 3.0 and 5.2 for rinsing and tablet groups, respectively) (10).

F regimes

The subjects in the rinsing group rinsed for 1 min under supervision with 10 ml of a neutral 0.05% NaF solution (228 ppm F). The subjects in the tablet group were requested to dissolve and move around a 0.5 mg F tablet (1.1 mg NaF with sorbitol and xylitol, Flux®, Alpharma). In both schools, F was taken every school day at least 1–2 h after breakfast for 3 weeks.

Plaque sampling

Four plaque samples were collected with blunted chisels on each occasion; one from the cervical thirds of the vestibular surfaces of the maxillary central incisors and mandibular 1st molars. The samples were placed in the lids of 0.2 ml Eppendorf tubes that were immediately closed, centrifuged and kept frozen at −18°C until analyzed. Sampling was performed 2–4 h after meal at baseline (day 0) and on the first and last school days of three consecutive weeks (days 4, 7, 11, 14, and 18). Samplings on days 7 and 14 were performed about 70 h after rinse or tablet use, and samplings on days 4, 11, and 18 about 22 h after.

F analysis

The F content of each sample was extracted by 10 µl of 0.25 *N* perchloric acid at room temperature for 2 h. The solution was neutralized with 10 µl of 0.25 *N* sodium hydroxide dissolved in 0.37 *N* sodium acetate buffer (pH 5.2) and centrifuged (11). The F content of two 1-µl drops of each supernatant was determined by the 'inverted' under-oil technique of Vogel *et al.* (12). The standard curve was log-linear above a concentration of 0.02 ppm F and the mean difference between parallels was 1 millivolt (mV). Re-analysis was performed at differences exceeding 5 mV.

Protein analysis

The protein content of each sample was analyzed *in duplo* for quantifying the plaque mass. The analysis was carried out by the BCA Protein Assay (Pearce Chemical Company, Rockford, IL, USA) after digestion in 4 *N* sodium hydroxide for 30 min at 90°C and neutralization in 4 N hydrochloric acid at room temperature (11). The mean coefficient of variation in protein content between parallels was 4.7%. Samples with less than 10 µg of plaque protein were excluded (13).

F amount assessment and statistical procedures

The mV-readings from the electrodes were converted to ng of F and related to µg of protein in the samples, assuming that 50 µg of protein corresponds to 1 mg of plaque (11). Results are presented in ppm (ng F/mg plaque wet weight) in order to facilitate comparison with other studies. Owing to high variances in F levels, median values and non-parametric tests were used. The differences in plaque F concentrations at baseline and on subsequent days were tested statistically. A *P*-value of less than 0.05 was considered significant.

Results

Subjects

Nine of the 42 subjects were not present on all school days and therefore excluded. Two subjects had too little plaque (<10 µg protein) in most samples and were also excluded. Another 13 of the 744 samples from the remaining 31 subjects were excluded for the same reason.

In the rinsing group, the majority of parents reported that their child was using F toothpaste daily, whereas these relationships were reversed in the tablet group (Table 1). F toothpaste usage and parents' income were related (Spearman's $r = 0.74$, $P < 0.01$).

Plaque F concentrations

At baseline, a wide range of plaque F levels was noted among subjects (Fig. 1). In addition, plaque samples from the same subject sometimes showed a high coefficient of variation (range 22.6–144.1%, mean 77.6%), although there was no consistent difference between incisor and molar plaques (Table 1). Toothpaste users from both groups showed higher plaque F levels than non-users ($P < 0.01$, Mann-Whitney U-test). Partly for this reason, the rinsing group showed 3–4 times higher median F levels than the tablet group ($P < 0.01$, Mann-Whitney U-test). However, even among non-users, baseline values tended to be higher within this group (Table 1).

Both programs increased plaque F levels (Fig. 1). When programs had been followed for 4 days and plaque was collected some 20 h later, median F values had tripled; from 9.1 to 27.3 in the rinsing group and from 2.5 to 8.0 in the tablet group (Table 1). During weekends, when F had not been used for about 70 h, values declined towards baseline levels. This decline was statistically significant from day 4 to day 7 for the rinsing group and from day 11 to day 14 for both groups ($P < 0.05$; Wilcoxon signed-rank test). The same changes can be seen when the toothpaste users were excluded (Fig. 2).

Table 1. Baseline plaque fluoride concentrations in ppm, median (range)

	Rinsing group Fluoride toothpaste			Tablet group Fluoride toothpaste		
	Users $n = 9$	Non-users $n = 6$	Both $n = 15$	Users $n = 4$	Non-users $n = 12$	Both $n = 16$
Incisor*	11.1 (1.3–38.6)	5.9 (0.4–14.7)	9.4 (0.4–38.6)	14.0 (5.3–50.1)	2.2 (0.2–8.9)	2.9 (0.2–50.1)
Molars**	14.1 (1.9–71.3)	4.2 (1.2–23.2)	9.2 (1.2–71.3)	6.1 (0.6–126.7)	1.3 (0.2–8.0)	2.1 (0.2–126.7)
All sites	12.6 (1.3–71.3)	5.7 (0.4–23.2)	9.2 (0.4–71.3)	8.4 (0.6–126.7)	2.0 (0.2–8.9)	2.4 (0.2–126.7)

Calculations based on a plaque protein content of 5%.
* $P < 0.05$; ** $P < 0.01$ by Mann-Whitney U-test.

The 9 F toothpaste users in the rinsing group showed values significantly below baseline on day 14 when the toothpaste had been out of use in this period and the rinse had been out of use during the weekend ($P < 0.01$, Wilcoxon signed-rank test; data not shown).

Discussion

The present study was designed to evaluate conventional topical F programs primarily by their effects on plaque F levels in disadvantaged children assumed to have a low F exposure. However, it appeared that the test subjects were of a more mixed socio-economic status, and more exposed to fluoridated dental products than anticipated. The caries prevalence, particularly in the permanent dentition, was found to be low and in line with data from the late 1980s (1, 3) as well as with recent data (2). A mean per capita sugar consumption of only 3 kg in Bangladesh may be of importance in this respect (14). Therefore, school-based F programs in these areas and for this age group do not seem to be indicated at present. Only a long-term trial in a caries active group can give an answer to the question of the preventive effectiveness of the programs. The discussion will therefore focus on the plaque F data.

For practical reasons, the topical treatments were restricted to school days. Since F levels were so much reduced after the weekends (Fig. 1), the present study supports the importance of daily supply (15, 16).

Plaque wet-weight could not be obtained under field conditions, so plaque amounts were assessed by quantitative protein analysis. Using this method, plaque from Norwegian orthodontic patients was found to contain an average of 50 μg of protein per mg wet-weight using the same analytical method and protein standard (11). However, as sucrose increases plaque volume (17), the low sucrose diet generally consumed in Bangladesh may have given a higher protein wet-weight ratio in the present study and a certain underestimation of plaque F levels.

The under oil technique developed by Vogel et al. (12) offers an opportunity for quantitative analysis of small F amounts with minimal F contamination from the environment. The technique seemed to function well, since values of parallel samples were close, and a log-linear relationship

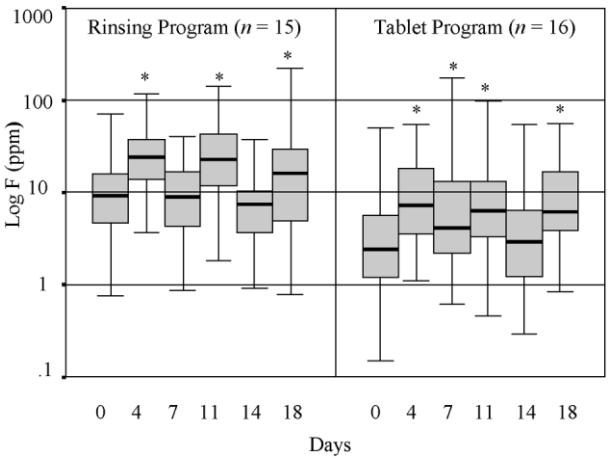

Fig. 1. Median ppm plaque fluoride levels (in log scale) in rinsing and tablet groups based on individual medians. * ($P < 0.05$) denotes higher than baseline, Wilcoxon signed-rank test.

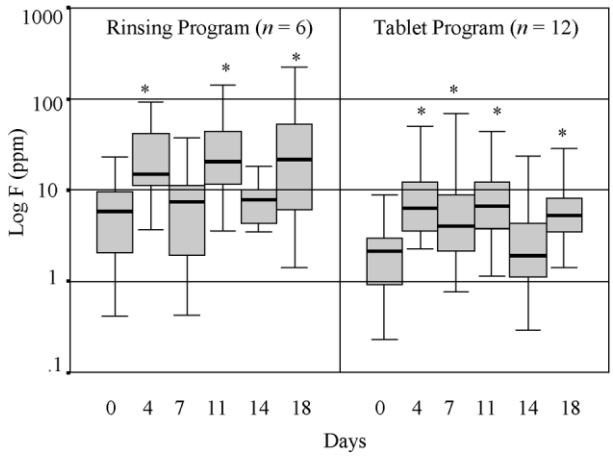

Fig. 2. Median ppm plaque fluoride levels (in log scale) in fluoride toothpaste non-users based on individual medians. * ($P < 0.05$) denotes higher than baseline, Wilcoxon signed-rank test.

between mV and ppm was observed down to 0.02 ppm F (18).

The F concentration of a topical agent provides the driving force for F to pass into the deeper plaque layers adjacent to the tooth (6). We used a NaF tablet containing 0.5 mg F and 10 ml of a 0.05% NaF rinse (2.3 mg F) on a daily basis. However, in both programs, the median baseline plaque F levels increased about 3-fold after 4 days of continuous use. Because of the difference in baseline plaque F levels between the rinsing and tablet groups, a direct comparison between the two programs does not seem warranted. However, based on available data (19, 20) total F exposures in the two groups may appear fairly equivalent. The maximum saliva level at the test sites can be estimated to be some 2–4 times higher in the rinsing group, but would presumably last some 3–5 times longer in the tablet group. Higher amounts of swallowed and re-secreted F from tablets than from the rinse would presumably be insignificant (21).

All sampling sites represent slow salivary clearance areas within the mouth (19, 22) and free smooth surfaces with relatively high caries risk (23). Even for these surfaces, plaque F levels on each sampling occasion varied considerably among subjects (Fig. 1) as well as within subjects. Other studies also report high variances within (24) and between (25) subjects.

The mean baseline plaque F concentrations recorded in the Bangladeshi groups were close to the range reported in the early literature (26), but high compared to more recent data (7, 12, 27). This seemed in part related to the use of F toothpaste by almost one half of the children (Table 1). However, those not using F toothpaste also showed a fairly high plaque F level. The first author found about one half of this level when samples, collected from Brazilian children of similar age, were analyzed by the same methods (10). It cannot be excluded that the higher plaque F in Bangladeshi children reflects a higher F level in the diet together with local tooth cleaning habits with sand, ash and charcoal (3). Based on observations on the relationship between plaque pH and F content (11, 28), high levels may also reflect a prolonged F retention in Bangladeshi children due to weaker acidogenicity in their plaques, as could be suspected from a much lower DMFT value in the Bangladeshi than in the Brazilian group (10).

A recent study showing that plaque F retention is related to its calcium content (29) also shows that loss of plaque F is a slow process. An average of three-quarters were lost between 1 and 12 h after use of a 1000 ppm dentifrice (29). Our samples were collected 20–22 h after the last topical treatment. Considerably lower values after a weekend than after 1 day are in accordance with the findings of Geddes & McNee (5).

In conclusion, our findings demonstrate fairly high baseline plaque F levels in Bangladeshi children, which could in part be attributed to F toothpaste usage. Both programs increased the plaque F levels. When F exposure had been stopped at the weekends, values declined towards baseline levels in 3 days.

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