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Fluorosis risk from early exposure to fluoride toothpaste

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Abstract – Swallowed fluoride toothpaste in the early years of life has been postulated to be a risk factor for fluorosis, but the epidemiological evidence is weakened by the fact that most of the relevant studies were done in developed countries where an individual is exposed to multiple sources of fluoride. *Objectives:* To quantify the risk of fluorosis from fluoride toothpaste in a population whose only potential source of fluoride was fluoride toothpaste. *Methods:* Case-control analyses were conducted to test the hypothesis that fluoride toothpaste use before the age of 6 years increased an individual's risk of fluorosis. Data came from a cross-sectional clinical dental examination of schoolchildren and a self-administered questionnaire to their parents. The study was conducted in Goa, India. The study group consisted of 1189 seventh grade children with a mean age of 12.2 years. *Results:* The prevalence of fluorosis was 12.9% using the TF index. Results of the crude, stratified, and logistic regression analyses showed that use of fluoride toothpaste before the age of 6 years was a risk indicator for fluorosis (OR 1.83, 95% CI 1.05–3.15). Among children with fluorosis, beginning brushing before the age of 2 years increased the severity of fluorosis significantly ($P<0.001$). Other factors associated with the use of fluoride toothpaste, such as eating or swallowing fluoride toothpaste and higher frequency of use, did not show a statistically significant increased risk for prevalence or severity of fluorosis. *Conclusions:* Fluoride toothpaste use before the age of 6 years is a risk indicator for fluorosis in this study population.

Key words: fluoride toothpaste; fluorosis; Goa, India; risk indicator

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Epidemiological studies in North America have shown that the prevalence of dental fluorosis has increased since the 1930s and 1940s (1–3). The increase in fluorosis is principally in the milder forms, though there has also been some increase in more moderate grades (3, 4), and is more pronounced in fluoride-deficient communities than in those with optimal or above optimal water fluoride concentration (1, 3, 5–8). Numerous studies have confirmed that fluoridated water and fluoride supplements are risk factors for dental fluorosis. Fluoride toothpaste use in children during the ''critical period'' of tooth development (birth to 6 years of age) has also been suggested as a risk factor for fluorosis (9–14), but the epidemiological evidence, while suggestive, is inconclusive (15–17). There are no studies, to date, that have looked at the risk of fluorosis when fluoride toothpastes are the sole source of fluoride.

Fluoride toothpaste, gels, and mouthrinses intended for topical use can inadvertently be ingested. Children under the age of 6 years can swallow up to 25–33% of the fluoride toothpaste used at each brushing because their swallowing reflex is not fully developed (18–24). Studies of fluoride absorption from ingested toothpaste conclude that bioavailability is high (>96%), and that absorption is essentially total (25–27). Other factors that strengthen the likelihood of fluoride toothpaste being a risk factor for fluorosis are: (a) more than 90% of children begin toothbrushing before the age of 2 years (9, 28–31); (b) a large number of children are not supervised while brushing their teeth (31, 32); and (c) some children use upwards of 0.5 g (0.5 mg

F) of toothpaste at each brushing (30, 32). Swallowed toothpaste can contribute up to 0.2–0.3 mg F per day to a child's daily fluoride intake (15, 33), which can clearly increase the risk of fluorosis.

The present study was designed to investigate the association between fluorosis and use of fluoride toothpaste during the critical period of tooth development in 12-year-old children in Goa, India. The potential source of fluoride in these children was fluoridated toothpaste. The drinking water supply fluoride ranges from 0.05 to 0.1 mg/L F; processed foods and beverages are not common; and fluoride supplements, gels and rinses are not available. Some fluoride may be present in the diet, mainly in fish and tea. Most Goan children below the age of 6 years do not usually drink tea; but, if they do, about three-fourths of the cup is usually milk. Fluoride from the diet is thus unlikely to affect fluorosis. In India toothpaste use is more common in higher socio-economic groups, so the study was centered in the private schools.

Approval for this study was received from the Institutional Review Board at the School of Public Health, University of Michigan, prior to its commencement.

Methods

This study tested the primary hypothesis that fluoride toothpaste use before the age of 6 years increases an individual's risk of fluorosis. Caries and oral hygiene were also assessed in the study, but those results are not presented in this report. Data came from a cross-sectional survey, consisting of a clinical dental examination of children and a self-administered questionnaire to their parents. Eleven of the most affluent private schools in the cities of Margao, Mapusa, Panjim, and Vasco-da-Gama were selected to take part in the study. The criteria used to select the schools were the tuition charged, the academic record (high pass percentage at the Secondary School Leaving Certificate Examinations from 1989–1994), and the examiner's knowledge of the more affluent schools in each city. In the selected schools, all seventh grade children present on the day the examinations were scheduled were examined.

Data were collected in the schools by one examiner (AKM), assisted by a recorder. Children were examined using a mouth mirror and explorer while seated in a field dental chair, in natural daylight. The buccal and occlusal surfaces of all permanent teeth were scored for fluorosis using the modified Thylstrup-Fejerskov (TF) index (34). Teeth were cleaned using cotton wool and gauze, and allowed to air dry for approximately 1 min before scoring for fluorosis. The scores were entered directly into a laptop computer using a direct data entry program in Epi-Info version 6 (35) developed by the examiner. To permit calculation of the intra-examiner reliability 5% of the children were re-examined. A month before the beginning of the field examinations, the examiner (AKM) had trained to use the TF index with Dr Ole Fejerskov, one of the original developers of the index.

A questionnaire, completed by parents of the children, included questions about demographic information, history of fluoride toothpaste use, brushing habits before the age of 6 years and at the present time, type of diet, and consumption of tea. The questionnaire was distributed only once, and no attempt was made to send home a second questionnaire to non-responders because, at most, only one child in each classroom had not returned the completed questionnaire. To ensure reliability of the questionnaire data, it was re-administered to a random 10% of the study group.

Goa has three main sources of water supply that have never been fluoridated. Two samples of water from each source at different points in time, January 1992 and January 1993, were tested at the Ann Arbor Water Treatment Plant using the Orion ion specific electrode. The water fluoride levels ranged from 0.05 to 0.1 mg/L F.

Statistical analyses were carried out using Epi-Info version 6.0 and the SAS system. Epi-Info was used first to derive or code new variables. Categorical variables such as frequency of toothbrushing at different ages, amount of toothpaste used, diet, and drinking tea were dichotomized. Epi-Info was also used to obtain descriptive statistics and frequency distributions of all the variables in the dataset. A new dataset was made using Epi-Info so that it could be exported into SAS for further analysis.

Pearson and Spearman correlation coefficients were used to test for correlations between scaled outcome and predictor variables, and between predictor variables; crude odd ratios (OR) with 95% confidence intervals (CI) were computed to measure the association between dichotomous outcome and predictor variables. Chi-square tests and Mantel-Haenszel chi-square (M-H) tests were used to determine whether the observed differences between outcomes and predictor variables were statistically significant. The M-H technique was also used to evaluate for confounding factors. The Bres-

low-Day chi-square was used to test for interactions between predictor variables. Observed interactions were further analyzed using stratified estimates (stratified ORs).

Finally, each hypothesis was tested separately using multiple logistic regression for dichotomous outcomes and multiple linear regression for continuous or scaled outcomes. Multiple models were generated and a final model was selected to explain the relationship between the outcome (dependent) variable and predictors (independent) variables. The model usually contained or controlled for all potential confounders or other risk indicators such as diet, source of water, place of residence before the age of 6 years, and drinking tea for the fluoride hypothesis. The final model also included explanatory variables such as gender and parent's education, and best explained the variability in the data.

Results

Participation

A total of 1250 of the 1276 seventh grade children in 11 of the more affluent schools in Goa were examined. The completed questionnaire was returned for 1189 children, with a mean age of 12.2 years, a response rate of 95.1%. Non-participation was due to absence from school on the day of the examination, or failure to return a completed questionnaire. The gender distribution of the study group was 58% males and 42% females.

Data reliability

The percentage agreements for the surface TF scores and the questionnaire were 95% and 82% respectively. The kappa value for the presence or absence of fluorosis was 0.75.

Sources of fluoride

Only 8.2% of the study group reported using fluoride toothpaste. In 89.3% of the study subjects' diet, fish was common, and 67% drank tea, with about half of these drinking one cup or less per day. In two-thirds of the children that drank tea, half the cup was reported to be milk.

Toothbrushing habits

Age at which toothbrushing began, method of toothbrushing, frequency of toothbrushing, and amount of toothpaste used are some of the toothbrushing characteristics this study examined. In 45% of these children toothbrushing began before the age of 2 years, and an equal percentage began between 2 and 4 years. A few parents (2.5%) responded that they did not remember when toothbrushing began. Parents reported that most children began brushing their own teeth about 2 years after toothbrushing by a caregiver began. The Pearson correlation coefficient between age at which toothbrushing initially began and age at which children began brushing their own teeth was 0.72 ($P<0.001$). Only 5.7% of the children reportedly started brushing their own teeth before the age of 2 years. The common method of cleaning the teeth when ''toothbrushing'' was first initiated was by using a toothbrush (81.6%). Other methods reported were using a parent's finger, 17.5%, or a cloth, 0.3%. Ninety-three percent of the parents reported using toothpaste when they first began brushing their child's teeth and 83% supervised their children's toothbrushing.

An interesting trend was seen in the frequency of toothbrushing at different ages in this study group (Table 1). Before the age of 2 years brushing once a

Table 1. Percentage distribution of daily frequency of toothbrushing at different ages. Fluoride toothpaste study, Goa, India, 1995

Frequency	Before 2 years	2–4 years	4–6 years	Now
Once a day	70.6%	78.6%	68.5%	53.3%
Twice a day	10.9%	17.8%	30.0%	44.1%
More than twice a day	0.8%	0.7%	0.9%	2.4%

Table 2. Stratified odds ratios for fluorosis and fluoride toothpaste association. Fluoride toothpaste study, Goa, India, 1995

Stratifying variable	*N*	Odds ratio	95% confidence interval
Frequency of toothbrushing before 2 years	909	1.62	(0.90–2.87)
Frequency of toothbrushing between 2 and 4 years	1075	1.73	(1.02–2.94)
Frequency of toothbrushing between 4 and 6 years	1099	1.73	(1.01–2.95)
Starting brushing before 2 years	1054	1.77	(1.03–3.05)
Amount of toothpaste used	1097	1.74	(1.02–2.97)
Eating toothpaste	1049	1.73	(1.00–3.00)
Swallowing toothpaste	1025	1.79	(1.03–3.11)
Drinking tea	1075	1.87	(1.10–3.19)
Residence outside Goa	1105	1.69	(0.98–2.87)
Mother's education (college education or less)	1105	1.75	(1.03–2.97)
Child's gender	1105	1.71	(1.01–2.92)

day was the favored frequency (70.6%), with only 10.9% of the children brushing their teeth twice a day. As the child got older the frequency of brushing increased with 44.1% of the children reportedly brushing their teeth twice a day at age 12. Brushing once a day was still the prevailing (53.3%) daily frequency of brushing reported. Less than 1% of the children brushed their teeth more than twice a day.

To describe the amount of toothpaste used per brushing, parents were asked to circle the diagram that most closely resembled the amount of toothpaste put on the brush. About equal numbers of children, 44% and 40.8% respectively, reportedly used toothpaste amounting to half or 3/4 the length of the brush-head. The rest of the children were equally divided (7%) between using a pea size amount or a full brush length of toothpaste.

Other toothbrushing characteristics evaluated were swallowing toothpaste, eating toothpaste, not rinsing after toothbrushing, and not spitting after toothbrushing. Twenty-four percent of the children were reported to swallow toothpaste, 20.6% ate toothpaste, 1.3% did not rinse after toothbrushing, while 6% did not spit out after toothbrushing.

Fluorosis

The prevalence of fluorosis was 12.9%. Fluorosis severity scores ranged from 0 to 5. Seventy-five percent of the children who had fluorosis had a TF score of 1. The crude odds ratio for fluorosis in children using a fluoride toothpaste before the age of 6 years was 1.71 (CI 1.01–2.91). Table 2 gives the stratified odds ratios for the fluorosis and fluoride toothpaste association. After simultaneously controlling for other factors such as source of drinking water, drinking tea, diet consisting of fish, and having lived outside Goa at any time, logistic regression analyses (Table 3) showed the odds ratio for fluorosis from using a fluoride toothpaste before the age of 6 years was 1.83 (CI 1.05–3.15). Other factors associated with increased risk of fluorosis were male gender, drinking tea, and having lived outside Goa any time before the age of 6 years. Interaction terms were tested in the model, but these did not add to the model, nor were they significantly associated, so they were dropped.

Children who had lived outside Goa before the age of 6 years (n=121) had a higher risk of fluorosis (OR=2.27, CI 1.34–3.85); they were therefore dropped from the analysis, and the analyses rerun.

Table 3. Risk of fluorosis when using fluoride toothpaste. Logistic regression model, fluoride toothpaste study, Goa, India, 1995

Independent variable	Parameter estimate	Standard error	Pr> chi-square	Odds ratio	Confidence interval
Fluoride toothpaste	0.60	0.28	0.032	1.83*	(1.05–3.15)
Residence outside Goa	0.82	0.27	0.002	2.27*	(1.34–3.85)
Well water	0.36	0.23	0.121	1.43	(0.91–2.25)
Well and tap water	0.11	0.25	0.658	1.12	(0.68–1.82)
Tea	0.42	0.21	0.050	1.52*	(1.01–2.30)
Diet consisting of fish	−0.11	0.32	0.741	0.90	(0.48–1.68)
Gender (males)	0.54	0.20	0.007	1.72*	(1.56–2.54)

* Statistically significant odds ratios.

Table 4. Risk of fluorosis when using fluoride toothpaste for those who had always lived in Goa. Logistic regression model, fluoride toothpaste study, Goa, India, 1995

Independent variable	Parameter estimate	Standard error	Pr> chi-square	Odds ratio	Confidence interval
Fluoride toothpaste	0.60	0.27	0.028	1.81*	(1.07–3.08)
Well water	0.42	0.24	0.101	1.47	(0.93–2.33)
Well and tap water	0.06	0.27	0.826	1.12	(0.62–1.81)
Tea	0.42	0.23	0.070	1.52	(0.96–2.41)
Diet consisting of fish	−0.11	0.32	0.741	0.83	(0.43–1.60)
Gender (males)	0.41	0.21	0.047	1.51*	(1.01–2.26)

* Statistically significant odds ratios.

Table 5. Risk of fluorosis, age of commencement of toothbrushing, and frequency of toothbrushing before the age of 6 years. Logistic regression model, fluoride toothpaste study, Goa, India, 1995

Independent variable	Parameter estimate	Standard error	Pr> chi-square	Odds ratio	Confidence interval
Toothbrushing before 2 years	0.31	0.20	0.114	1.37	(0.92–2.02)
Frequency of toothbrushing	0.21	0.25	0.393	1.23	(0.76–2.01)
Amount of toothpaste used	0.11	0.20	0.564	1.12	(0.75–1.65)
Eating toothpaste	−0.20	0.25	0.413	0.82	(0.50–1.34)
Fluoride toothpaste	0.66	0.30	0.027	1.94*	(1.07–3.35)
Residence outside Goa	0.75	0.29	0.008	2.12*	(1.20–3.74)
Well water	0.26	0.25	0.308	1.29	(0.79–2.12)
Well and tap water	0.14	0.26	0.587	1.16	(0.69–1.91)
Tea	0.42	0.22	0.060	1.52	(0.99–2.34)
Diet consisting of fish	0.09	0.35	0.797	1.10	(0.55–2.17)
Gender (males)	0.57	0.21	0.007	1.77*	(1.17–2.67)

* Statistically significant odds ratios.

Table 6. Fluorosis severity, multiple regression model, Goa, India, 1995

Independent variable	Parameter estimate	Standard error	T statistic	Pr> |T|
Use of fluoride toothpaste	0.17	0.07	2.60	0.011*
Toothbrushing before 2 years	−0.58	0.15	−3.90	0.001*
Frequency of toothbrushing	−0.06	0.19	−0.32	0.743
Residence outside Goa	0.18	0.21	0.87	0.388
Well water	0.33	0.18	1.85	0.067
Well and tap water	−0.16	0.20	−0.84	0.405
Tea	0.22	0.16	1.38	0.169
Diet consisting of fish	0.46	0.25	1.83	0.070
Gender	−0.16	0.17	−0.95	0.344
Mother's education	0.13	0.34	0.39	0.695

F value=3.20, Pr>F 0.001, R-square=0.20
* Statistically significant at the $P<0.05$ level.

Table 4 reports the logistic regression analysis for those children who had always lived in Goa. Fluoride toothpaste and gender were still statistically significant risk indicators, while drinking tea did not show a statistically significant increased risk. The risk of fluorosis from the use of fluoride toothpaste for those children who had lived outside Goa before the age of 6 years was 5.42 (CI 1.35–21.79).

Tables 5 and 6 are the logistic and multiple regression models for other factors associated with the prevalence and severity of fluorosis. Beginning brushing before the age of 2 years did not increase the prevalence of fluorosis but increased the *severity* of fluorosis significantly $P<0.001$ (Table 5). Other factors associated with the use of fluoride toothpaste, such as eating or swallowing it or higher frequency of use, did not show a statistically significant increased risk for prevalence or severity of fluorosis.

Discussion

The study was restricted to children from the higher socio-economic strata in Goa, because use of a fluoride toothpaste is not widespread in all sections of the society in India. The response rate of 95.1%, with no incentives offered to the study participants, was excellent. People were generally eager to take part in the study.

As with any study using a questionnaire, recall of the respondent is an issue. The children's parents were asked to recall behaviors of 6 to 12 years before. There probably was some difficulty in accurately recalling the past; however, any recall error is expected to have been random as the respondent was unaware of the ''disease'' status of the child. Additionally, the percentage agreement of 82% shows that the data from the questionnaire were reliable and consistent. The examiner (AKM)

was unaware of the child's fluoride exposure history during the dental examinations, thus ruling out examiner bias.

The study sample consisted of more boys than girls. This finding is consistent with the general population ratio and with that of children in schools across Goa (36, 37).

Toothbrushing in these children began later in life compared to developed countries where 52–83% children are reported to begin brushing their own teeth before the age of 2 years (1, 17, 29, 31, 32). For the esthetically important permanent anterior teeth, the critical period for developing fluorosis is between 20 and 30 months following birth (4, 38–41). Starting toothbrushing a little later may therefore be an advantage where fluorosis is concerned.

A pattern was also seen for the time period between the initiation of toothbrushing by a parent or a caregiver and the child beginning his or her own toothbrushing. The data suggest that the norm was for the child to take over toothbrushing 2 years after the practice was first begun. At the time of the study, the majority of these children were still brushing their teeth once a day. This finding suggests that although the study group had higher socio-economic status (SES), they were not ''dentally'' aware or conscious of appropriate and recommended dental behaviors.

Ninety-three percent of this study sample used toothpaste when they first began brushing their teeth. This result was expected as these children were of the higher SES group. Although about 50% of the brands of toothpaste on the Indian market contained fluoride, only 8% of the children were reported to have used them. The only explanation that can be offered for this finding is that the parents of these children were unaware of fluoride's role in preventing caries.

The fluorosis prevalence in this study sample was 12.9%. When compared to other non-fluoridated areas, the fluorosis prevalence in this study is similar to that found by Szpunar & Burt (2) and Ellwood et al. (42), but lower than that found in the studies of Woolfolk et al. (43), Pendrys & Katz (16), Ismail et al. (8), and Riordan & Banks (17). The indices used to measure fluorosis in most of these studies differed. Except for the Fluorosis Risk Index (FRI) used by Pendrys & Katz, the TF index used in the present study is the most sensitive fluorosis index. As such it would be expected that the prevalence of fluorosis in this study would be higher. The lower prevalence of fluorosis is because fluoride toothpaste was the only potential source of fluoride, compared to multiple sources of fluoride in the other populations studied. The prevalence of fluorosis in this study, when compared to others in which Dean's index has been used, is about twice as high; again most of this difference is because of the indices used (5, 6, 44, 45). When the TF index was more nearly equated with Dean's index by not including a TF score of 1, the fluorosis prevalence in the study group dropped to 3.3. This fluorosis prevalence is still lower than that seen in non-fluoridated communities in North America (4), as would be expected.

A comparison of fluorosis prevalence in other developing countries and this study group is restricted because there are no studies from these countries of fluorosis prevalence and severity in non-fluoridated communities. Previous studies in communities with low water fluoride levels (0.19–0.30 mg/L) in India have reported no fluorosis (46, 47). But at 1.1 mg/L 85% of the children had fluorosis (46). The water fluoride levels in the present study are lower (<0.1 mg/L) than that in the above studies, but the fluorosis prevalence is higher. Possible reasons for the difference in fluorosis prevalence of the magnitude seen at 0.30 mg/L and 1.1 mg/L fluoride in the Subbareddy & Tewari report could be either the misdiagnosis of fluorosis at milder levels even though the teeth had been dried, or the fluorosis seen in Goa could be due to a factor other than water fluoride. In the Goan study the only potential source of fluoride was toothpaste. Children in the other Indian studies were of lower SES and probably did not use toothpaste. Fluorosis from use of fluoride toothpaste was therefore not a risk factor in their studies.

Fluorosis severity in this study group ranged from 1 to 5 on the TF scale. Seventy-five percent of the children who had fluorosis exhibited only the mildest form (TF 1), meaning that fluorosis was not apparent without first drying the tooth. This finding is consistent with that of Riordan & Banks (17) from Australia and Ellwood et al. (42) in Brazil.

The crude risk of fluorosis if a child had used fluoride toothpaste before the age of 6 years was 1.71 (CI 1.01–2.91). This association essentially did not change when stratified by other potential sources of fluoride and factors associated with toothbrushing. After simultaneously controlling for other potential sources of fluoride and confounding factors using logistic regression, the relationship still held with an odds ratio of 1.83 (CI 1.05–3.15). Even after children who had not lived in Goa

before the age of 6 years had been dropped from the logistic regression analyses, the risk of fluorosis was 1.81 (CI 1.07–3.08). The conclusion which can be drawn is that early use of fluoride toothpaste increases the risk of fluorosis. This conclusion is consistent with other reports (10, 12, 17), although the magnitude differed from those studies by Osuji et al. (9), Pendrys & Katz (16), and Lalumandier & Rozier (14). In these studies 99% of the children reported using fluoride toothpaste. There were essentially no comparison groups that had not used fluoride toothpaste or were not exposed to fluoride in some form or other.

In the children who had lived outside Goa sometime before the age of 6 years, the odds ratios for fluorosis and use of fluoride toothpaste were higher (5.42) and similar to those seen by Pendrys & Katz (16) and Lalumandier & Rozier (14) (3.6 and 3.0 respectively). These children had probably been exposed to fluoride from sources other than fluoride toothpaste such as fluoride in water. This result indicates the cumulative or additive effect of fluoride, increasing the individual's risk of fluorosis from any source when there is an increase in the number of sources of fluoride or increase in total fluoride intake.

In the present study, fluorosis risk was higher in males than females, a difference not previously reported. There were no obvious different cultural or behavioral factors between the males and females in this study. The literature supports the theory that fluorosis is more prevalent and severe in later developing teeth such as premolars and second molars (39, 48–50). Teeth in males develop a little later than in females, and as such could show a higher risk of fluorosis when compared to females.

The risk of fluorosis in children who had begun brushing before the age of 2 years was higher than the total study group, but this increased risk was not beyond that expected due to chance. However, among the children who had fluorosis, those who had started brushing before the age of 2 years had a significantly higher *severity* of fluorosis. This finding is supported by the literature, as younger children tend to swallow more toothpaste (20, 22–24). Other variables associated with toothpaste use that have been shown to increase an individual's risk of fluorosis, such as increased daily frequency of toothbrushing, reportedly eating toothpaste or swallowing toothpaste, did not increase the risk of fluorosis in this group. This was not an unexpected result because less than 10% of the children studied had used fluoride toothpaste, and there was little variability in their pattern of use. More important, when the crude association seen between use of fluoride toothpaste before the age of 6 years and fluorosis was stratified by the above variables, the association and risk of fluorosis did not change.

In summary, results of the crude, stratified, and logistic regression analyses showed that use of fluoride toothpaste before the age of 6 years is a risk indicator for fluorosis (OR 1.83, 95% CI 1.05–3.15).

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