

ORIGINAL ARTICLE

Differences in exposure and biological markers of fluoride among White and African American children

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Abstract

Objective: To determine differences in self-reported fluoride exposure and fluoride exposure biomarkers between two racial groups.

Methods: Questionnaires regarding fluoride exposure, urine and water collection kits were distributed to African American and White 7-14-year-old children. Children received a dental exam for fluorosis. Water, urine, and saliva were analyzed for fluoride content. Questionnaire responses and results of sample analyses were compared and observed differences were analyzed.

Results: 83 African American and 109 White children completed the study. Dental fluorosis was observed in 62.5 percent White and 80.1 percent African American children. Significant differences were found for fluorosis prevalence and severity between the groups ($P < 0.05$). Less African American children reported having used fluoride supplements in the past. White children began brushing their teeth at an earlier age. More White children visited a dentist for the first time before age 3. African American children reported currently using larger amounts of toothpaste. More Whites than African Americans had received topical fluoride treatments over the previous year. All of these differences were significant. Multivariate models showed that supplement use and amount of toothpaste used for brushing had significant associations to a child's fluorosis scores. Fluoride concentration of water and saliva was not different for the two groups; however, the fluoride content in urine was significantly higher in African Americans than in Whites [$P < 0.05$; $1.40 \pm$ standard deviation (SD) 0.65 ppm versus $1.08 \pm$ SD 0.28 ppm].

Conclusions: Differences in fluoride exposure between two racial groups were observed. These differences are complex and need to be better defined.

Introduction

Fluoride has been widely recognized as a safe and effective agent in the prevention of dental caries (1). It has been proposed that the marked reductions in caries prevalence and severity observed over recent decades in the United States are mainly due to the generalized use of fluorides. Current understanding of the mechanism of action of fluoride indicates that its major effect is posteruptive and topical, and that effectiveness depends on fluoride being present during caries formation and reversal (2). Differences in a subject's exposure to fluoride have been demonstrated to significantly influence its effectiveness (2).

Poor oral health disparately afflicts defined groups of the American population. Despite its overall reduction, dental

caries continues to be the most common infectious disease of childhood (3). Dental caries, while no longer pandemic, is endemic in specific individuals within populations, with children from racial minorities (among others) carrying a disproportionate burden of the disease. In addition, differences have been reported in the prevalence of other dental conditions, such as dental fluorosis, in racial and ethnic minority children (4,5). According to the US Surgeon General's Report in 2000, disparities can be observed not only in dental health outcomes, but considerable gaps in access to dental care also remain, especially among different ethnic/racial groups (6).

It has been proposed that the observed oral health disparities may be associated, among other factors, to differences in access and type of dental care, which may result in differences in fluoride exposure (3,7-11). Differences in factors that

strongly influence access to care and as a result may affect fluoride exposure, such as oral hygiene habits and dental knowledge, have been reported for racial and ethnic minorities (3,7-11). Disparities have been reported in the frequency of professionally applied fluoride and utilization of supplements, factors that also influence fluoride exposure, among different ethnic/racial groups (6-14). Nourjah and coworkers (13) found that children from racial minorities reported less use of dietary fluoride supplements. Aday and Forthofer (14) reported differences in the access to preventive dental care between Whites and other races. In their study, African American and Hispanic minority subgroups were less inclined to have private dental insurance coverage, to be knowledgeable about the reasons for using fluoride, to have visited a dentist in the past year, and Hispanics were more apt to have gone in response to symptoms rather than for preventive reasons. Therefore, the evaluation of the factors that influence fluoride exposure in children from varied racial and ethnic groups could be a reasonable approach to assess the role of fluoride in the public health prevention of caries.

In addition to assessing factors that influence fluoride exposure through retrospective questioning of subjects or prospective longitudinal follow-up, it has been proposed that measuring biomarkers could be a suitable approach to estimate past and present fluoride exposure (15-20). Fluoride levels in body tissues and fluids, i.e., plasma, urine, saliva, nails, or bone have been proposed as appropriate fluoride biomarkers (15-20). Dental fluorosis, a condition that arises from excessive fluoride ingestion during tooth development, has been proposed as a potential biomarker for past fluoride exposure. Although fluorosis development has been proposed to have a genetic component, it can also be partially considered the result of differences in fluoride exposure (21). Marked differences in fluoride exposure biomarkers, specifically in dental fluorosis, have also been described by a few investigators for different racial/ethnic groups (4,5).

Comprehensive data collection to study differences in exposure to fluoride among different racial groups would be a valuable step to better understand and begin to address some of the reasons for oral health disparities. Therefore, the overall goal of this investigation is to determine specific differences in exposure to fluoride through water, fluoride supplement use, professionally applied fluoride, and brushing habits. Differences in exposure between two racial groups will be associated to three fluoride exposure biomarkers: urine, saliva, and dental fluorosis.

Methods

Institutional Review Board approval was obtained prior to the initiation of the study. Local schools with similar numbers of African American and White children from similar income-level neighborhoods were selected for this study conducted in

Indianapolis, Indiana, USA, an optimally fluoridated community (water fluoride level adjusted to 1 ppm with a control range from 0.9 to 1.5 ppm). Schools were invited to participate based on a history of previous collaborations with the research team and matched for socioeconomic factors and for racial composition. Letters describing the study, informed consent statements and abbreviated medical history, demographic, and residency questionnaires were mailed to the parents of potential participants. All children in the age range from each school were invited to participate in the study. Investigators visited the participating schools to explain the purpose of the study and to answer questions. The medical history/demographic/residency questionnaire and informed consent were returned to the schools by parents and collected by the investigators to determine initial acceptance.

Potential participants were required to be between the ages of 7 and 14 with at least one erupted central incisor to examine for the presence or absence of dental fluorosis. Children that age were chosen because, in most cases, their central incisors have erupted, and that would allow us to use the presence of dental fluorosis as a biological marker for previous fluoride exposure. Participants also needed to have no factors in their medical history which could contraindicate collection of urine or saliva samples or receiving a dental exam; have their parents consent to their participation; have their parents' complete a "Fluoride Exposure" questionnaire (including information about diet, previous dental history, use of supplements); have lived in the same community since birth and report their race as non-Hispanic White or non-Hispanic African American.

"Fluoride Exposure" questionnaires, urine and water collection kits to use at home were distributed at the schools. Instructions were provided for the collection of samples and completion of questionnaires; children were asked to return them at their appointment. The "Fluoride Exposure" questionnaire employed is a modification of a questionnaire successfully used in previous studies (22-24). In those initial studies, the questionnaire was used to assess short-term recall of past fluoride exposure (3 to 6 months). Our team of investigators has used this modified questionnaire to assess longer-term recall of past fluoride exposure, similar to the recall required for this study (25). The questionnaire was modified to include questions regarding beverage consumption in addition to type of water consumed by children; current and past toothpaste use, and age at which brushing began, fluoride supplements use, dental visits and age of first dental visit, exposure to in office topical fluoride, and use of fluoride rinses. For each category, several examples were included. For instance, for topical fluoride treatments, gels, foams, and varnishes were described. For rinses, most commercially available products were listed and it was clearly stated that some specific products (i.e., mouthwashes) should not be considered in this category.

Incomplete or incorrect answers were clarified by telephone with the parents. Children were asked to bring with them to their appointment a water sample from their primary source of drinking water (community water, bottled, etc.). A water sample from each school was also collected and analyzed for fluoride.

The collection of saliva samples was performed in groups. Participants were asked to fast prior to saliva samples collection. They chewed a gum base and expectorated into a funnel placed in a graduated, plastic tube for a period of 5 min or until 1.5 mL of saliva had been collected and the flow rate (mL/min) was calculated. On the day of their appointment, panelists were asked to collect a urine sample from the first urine of the day and to bring the sample with them to this appointment. Saliva and urine samples were then frozen for later analysis.

Prior to the dental examinations, participants were asked to brush their teeth with a toothbrush and water. The fluorosis dental exams were performed by a single calibrated examiner using the Tooth Surface Index of Fluorosis (TSIF) developed by Horowitz *et al.* (26). A training and calibration session for this examiner was conducted prior to the initiation of the study. The examiner observed the buccal, lingual, and occlusal surfaces of permanent teeth, with the exception of the third molars, and a score was assigned to each surface. The dental fluorosis exams were performed using conventional portable dental equipment including portable dental chairs, lights, plane surface mirrors, and dental explorers.

At the time of examination and collection appointment, each panelist was assigned a study number. All samples and examination data were identified only by these numbers to ensure the blinding of the biological samples. The investigators followed strict procedures based on the Indiana University School of Dentistry (IUSD) infection control guidelines in order to minimize the risk of infection or cross contamination.

The bottled water brands most frequently reported by the participants were obtained. Tap and bottled water samples, urine, and saliva samples were analyzed for fluoride content. Comparisons were made among the two racial and age groups and differences were analyzed and possible changes in tendencies among the two races were assessed.

Fluoride analysis

Water samples were analyzed directly using a combination fluoride-specific electrode (Orion #96-909-00). Analysis of urine and saliva was conducted using a modification of the hexamethyldisiloxane (HMDS: Sigma Chemical Co., St. Louis, MO, USA) microdiffusion method of Taves as modified by Martinez-Mier *et al.* (27). Fluoride content of each sample was obtained by comparison of the millivolt reading of the sample to a standard curve prepared from the data for

diffused fluoride standard solutions analyzed at the same time. All samples were analyzed in duplicate at the IUSD fluoride laboratory by calibrated technicians.

Statistical analysis

Comparisons were made among the type of water consumed, fluoride supplement use, dental visits, and brushing habits reported in the "Fluoride Exposure" questionnaire. Yes/no questions were analyzed using chi-square tests. Urine and saliva fluoride data were analyzed using the Students' *t*-test. Fluorosis cases were defined as participants with two or more TSIF scores greater than 0. Prevalence of fluorosis was calculated for the two groups and then at the subject level. Severity was reported on the basis of the maximum score assigned to an individual. Fluorosis scores were used in multivariate analysis of variance (MANOVA) models to test for associations with reported fluoride exposure and to test for differences between groups. Specific fluoride exposure information was evaluated in order to correlate it to the fluorosis prevalence observed. Those exposure factors which had a significant effect on the severity scores were used to provide some insight on the past fluoride exposure of study participants. Before the analyses were performed, the data were examined to determine if they were normally distributed and had equal variances. Significance level was set at 0.05.

Results

Parents of 83 African American and 109 White children completed the fluoride exposure self-administered questionnaire, returned their children's urine and tap water samples, and had their children's saliva collected and dental exam performed. There were no statistical differences in mean age, gender, and years of residency in the community or socioeconomic status between the two groups.

We assessed intra-examiner reliability in our study by calculating the percentage of agreement for repeated examinations for the scores given to central incisors and first molars. It was 87.5 percent for central incisors and 82.3 percent for first molars, with Kappa values of 0.78 and 0.72, respectively.

Results from the dental fluorosis exams are presented in Figure 1. They showed that the prevalence in African American children (80.1 percent) was significantly higher than in Whites (62.5 percent) ($P < 0.01$). Of the 62.5 percent of the White children who presented with dental fluorosis upon examination, 41.3 percent had a maximum score of 1 and only 21.2 percent of the children had a maximum score of 2. Of the 80.1 percent of African American children who had dental fluorosis, a maximum score of 1 was assigned to 50.5 percent of the children, 15.4 percent were assigned a maximum score of 2, 1.5 percent had a maximum score of 3,

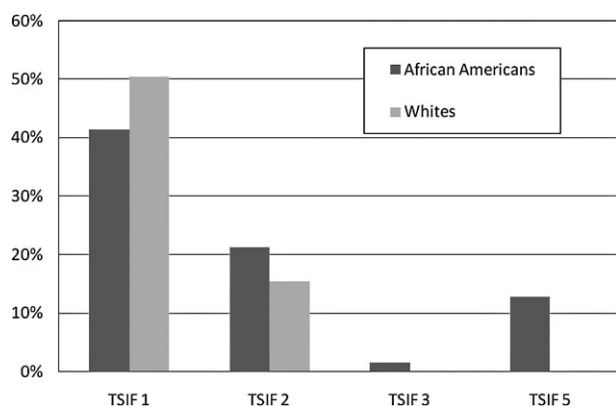


Figure 1 Maximum TSIF score assigned to children expressed as percentages.

and 12.7 percent were assigned the highest score of 5. Differences in severity were also statistically significant ($P < 0.001$).

Responses to the fluoride questionnaire are shown in Table 1. We assessed both past exposure, relevant to dental fluorosis development as well as the children's current exposure. For past exposure, African American children reported a lower percentage of having used fluoride supplements versus White children, 6.0 percent and 16.5 percent, respectively. White children also began brushing their teeth at an earlier age. African American children reported using larger amounts of toothpaste, 73.5 percent of the African American children used a full strip of paste covering the toothbrush, while only 55.0 percent of the White children used a similar amount. Of the White children, 68.8 percent had visited the dentist for the first time before age 3, while this was true for 45.8 percent of the African American children. Statistical chi-square analysis indicated that all the reported differences were significantly different ($P < 0.05$).

As for current exposure, similar numbers of children reported brushing their teeth using fluoridated toothpaste (86.2 percent and 81.9 percent). A larger number of White children had received topical fluoride treatments over the last year than African American children (54.1 percent and 34.9 percent, respectively). African American children reported using larger amounts of toothpaste, 95.2 percent of the African American children used a full strip of paste covering the toothbrush, while only 55.0 percent of the White children used a similar amount. Statistical chi-square analysis indicated that all the reported differences were significantly different ($P < 0.05$).

The mean fluoride concentration of tap water was not different for the two groups (0.072 ppm for Whites and 0.077 for African Americans) ranging from 0.16 to 2.22 ppm. Thirteen children were exposed to water below optimal concentrations (below 0.9 ppm), while seven children were exposed to water above optimal concentrations (above 1.5 ppm). None of the children who currently drank water with fluoride content higher than recommended had a maximum TSIF score of 3 or 5.

The majority of children used solely tap water (84.42 percent of Whites and 74.71 percent of African Americans), bottled water was used by 9 (8.25 percent) Whites and by 10 (12.04 percent) African American children. Both types of water were used by 8 (7.33 percent) White and 11 (13.25 percent) African American children. No differences in other types of beverages consumed by participants (sodas, juices, energy drinks) were found.

Results of the multivariate model (MANOVA) showed that supplement use (P -value < 0.02) significantly increased mean severity score when adjusted for other indicators. The risk indicator of amount of toothpaste used for brushing (P -value < 0.02) was also significantly associated with severity scores. Mean values for water, urine, and saliva samples are presented

Table 1 Self-Reported Fluoride Exposure

	Whites N (%)	African Americans N (%)
Historical fluoride exposure		
Children who were given fluoride supplements in the past	18 (16.5)	5 (6.0)*
Children who had a first dental visit <3 years	75 (68.8)	38 (45.8)*
Children who received topical F over the last year	59 (54.1)	29 (34.9)*
Children who reported to currently use > half brush covered of toothpaste <3 years	60 (55.0)	61 (73.5)*
Children who started to brush at <3 years of age	88 (79.8)	58 (69.9)
Children who started own brushing at <3 years	42 (38.5)	30 (36.1)
Current fluoride exposure		
Children who currently used fluoridated toothpaste	94 (86.2)	68 (81.9)
Children who reported to currently use > half brush covered of toothpaste	60 (55.0)	79 (95.2)*
Children who reported currently toothbrushing >2 day	90 (82.6)	64 (77.1)
Children who currently used fluoride rinse	17 (15.6)	30 (36.1)*

* Difference is statistically significant ($P < 0.05$).

Table 2 Mean and Range of Fluoride Concentration of Water, Urine, and Saliva Samples

	Whites Mean F ppm (\pm SD) Range F ppm	African Americans Mean F ppm (\pm SD) Range F ppm
Water	0.75 (0.18) 0.16-2.26	0.78 (0.10) 0.22-1.98
Urine	1.08 (0.28) 0.02-2.60	1.40 (0.65)* 0.24-3.07
Saliva	0.015 (0.002) 0.001-0.10	0.015 (0.001) 0.001-0.15

* Difference was statistically significant ($P < 0.05$).

SD, standard deviation.

in Table 2. The fluoride content in urine was significantly higher in African Americans than in Whites ($P < 0.05$). However, the fluoride content in saliva was not statistically different. No associations were found between water fluoride content and urine fluoride content.

Discussion

Our results support the findings of studies that have indicated marked differences among White and minority children with regard to self-reported fluoride exposure (6-14). Decreased exposures to fluoride in the forms of professional fluoride applications, fluoride supplement use, and frequency of brushing were observed for African Americans. The only exception to the differences observed between the groups in this study was that African American children reported a more frequent use of fluoride rinses than Whites. These results are in agreement with previous reports that have pointed out that more African Americans seem to use rinses than Whites or Hispanics (28).

The fact that African American children reported a lower use of fluoride supplements than White children can be considered an indicator of less access to dental care and is also consistent with previous reports (13). Differences in children who had not received topical fluoride treatments over the last year are also an indication of lower access to care in the African American group. Also consistent with this trend is the fact that White children in our study appeared to have visited the dentist at an earlier age than African American children. Our results are therefore in agreement with the study conducted by Aday and Forthofer (14) that concluded that Blacks and Hispanics were less apt to have access to dental care.

In this community, there appeared to be similar numbers of children that reported brushing their teeth using fluoridated toothpaste; however, a closer examination to toothbrushing habits revealed parents of White children began brushing their children's teeth at an earlier age and used an amount of toothpaste more in agreement with current fluo-

ride recommendations for children (29). This may be an indicator of a lack of dental knowledge relative to fluoride use among African Americans as suggested by the results of Aday and Forthofer (14) and by Edelstein in his recent review of oral health disparities (9).

Choice of water source did not seem to be affected by race in this study. Contrary to the studies that have indicated that certain ethnic or racial groups have different preferences in water intake (30), children in our study did not report a difference in bottled water use and were exposed to water with similar concentrations of fluoride. The range of fluoride in tap water, although not different for the two groups, showed a significant range of 0.16 ppm to 2.26 ppm. Further analysis of questionnaire responses revealed that some of the children were residents of a community that is part of the Indianapolis metropolitan area, but that has a natural fluoride concentration in the water of 2.00 ppm. Some others also reported using well water that seemed to contain minimal fluoride.

In addition to assessing exposure through self-reported use of fluoride products, a second approach was considered in this study. To our knowledge, no other study has reported comparative values for urine and saliva used to assess differences in fluoride exposure among different racial groups, so comparisons with our data were not possible.

Both saliva and urine concentrations of ionic fluoride in humans are considered reasonable indicators of current fluoride exposure for populations (15-20). Based on the understanding of fluoride pharmacokinetics and metabolism, we may conclude that urine fluoride concentration is a better indicator of fluoride ingestion, while salivary concentration appears to be a better indicator of recent topical fluoride exposure (15).

Dental fluorosis presence is considered a potential biomarker for higher than optimal past fluoride exposure in populations (21). Based on this assumption, our results seem to indicate that African American children ingested larger amounts of fluoride at a younger age. At ages 7-14, it is not expected that children in our study could still present reflex swallowing. It is therefore unclear if the report of current use of greater amounts of toothpaste on the brush or increased use of fluoride rinses would result in appreciably higher concentrations of ingested fluoride, or be the cause for the higher fluoride concentrations in the urine. This ingestion may be related to other sources of fluoride, not covered by our questionnaire.

The amount of fluid consumed (water or otherwise) was not reported through our questionnaire. Fluoride concentrations in urine and saliva are both influenced by the amount and frequency of fluid consumption. Therefore, we may not conclude whether the higher level of fluoride in urine of African American children could have been due to consumption of more water (other fluids and beverages). We could

infer that this increased fluoride ingestion was present in the children participating in the present study when they were younger, as indicated by the higher prevalence of dental fluorosis observed for African Americans. Several reports have indicated that there is in fact an increased prevalence of dental fluorosis in African American children (4,5).

Due to the nature of our study and our recruitment strategies, which aimed at over representing children with dental fluorosis, our prevalence results should not be considered representative. The study was designed to use fluorosis presence as a potential biomarker for past fluoride exposure. It should be mentioned that several of the questions asked parents to recall the child's practice before age 3. Recall bias is always an issue when questionnaires are used to collect information. However, by conducting a dental exam, we are assessing the prevalence of dental fluorosis as a biomarker to potentially validate those responses that inquired about past fluoride exposure in the questionnaire. In our questionnaire, we were also inquiring about current fluoride exposure, which does not require parents to recall practices that happened years ago. However, current fluoride exposure is not necessarily indicative of past exposure nor is a risk for erupted teeth.

Although fluorosis presence cannot solely be explained by past fluoride intake, our results showed a significant effect on the fluorosis severity score when children reported using larger amount of toothpaste and supplements, which points to the value of using fluorosis as a biomarker for past fluoride exposure. Numerous previous studies have shown significant associations between these factors and increased fluorosis risk (5,20,21).

The results of our study lead to the conclusion that there are indeed differences in fluoride exposure and ingestion among different ethnic/racial groups, and that these differences are complex, need to be better defined, and may only be partially responsible for differences in dental caries and fluorosis observed among different racial/ethnic groups. It would appear that patterns of fluoride intake and exposure in African American children in our study are conducive to maximizing fluoride's benefits (mostly its topical effects, since they reported using larger amounts of toothpaste for brushing and rinsing more frequently). However, these patterns also seem conducive to increasing the risk for the development of dental fluorosis. It would also appear that White children have greater access to professionally applied fluorides and seem to have fluoride exposure patterns (smaller amount of toothpaste used for brushing and less frequent use of rinses) that are conducive to reducing their risk of developing dental fluorosis. Future studies should aim at further characterizing these differences and assessing their specific roles as risk factors for the observed differences in dental caries and fluorosis observed among different racial groups.

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