Cancer Causes Control (2006) 17:421–428
DOI 10.1007/s10552-005-0500-6

ORIGINAL PAPER

Age-specific fluoride exposure in drinking water and osteosarcoma (United States)

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Received: 24 July 2005 / Accepted: 7 November 2005

Abstract
Objective We explored age-specific and gender-specific effects of fluoride level in drinking water and the incidence of osteosarcoma.
Methods We used data from a matched case–control study conducted through 11 hospitals in the United States that included a complete residential history for each patient and type of drinking water (public, private well, bottled) used at each address. Our analysis was limited to cases less than 20 years old. We standardized fluoride exposure estimates based on CDC-recommended target levels that take climate into account. We categorized exposure into three groups (<30%, 30–99%, >99% of target) and used conditional logistic regression to estimate odds ratios.
Results Analysis is based on 103 cases under the age of 20 and 215 matched controls. For males, the unadjusted odds ratios for higher exposures were greater than 1.0 at each exposure age, reaching a peak of 4.07 (95% CI 1.43, 11.56) at age 7 years for the highest exposure. Adjusting for potential confounders produced similar results with an adjusted odds ratio for males of 5.46 (95% CI 1.50, 19.90) at age 7 years. This association was not apparent among females.
Conclusions Our exploratory analysis found an association between fluoride exposure in drinking water during childhood and the incidence of osteosarcoma among males but not consistently among females. Further research is required to confirm or refute this observation.

Keywords Osteosarcoma · Fluoride · Fluoridation · Case–control

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Introduction

Osteosarcoma is a very rare primary malignant tumor of bone. Although uncommon, primary malignant bone tumors comprise the sixth most common group of malignant tumors in children and the third most common malignant tumor for adolescents, with an annual incidence rate of 5.6 per million for Caucasian children under 15 years old [1]. Osteosarcoma is the most common tumor of bone and for patients less than 20 years old more than 80% of these tumors tend to occur in the long bones of the appendicular skeleton which are undergoing rapid growth [2]. The incidence of osteosarcoma is slightly higher in males than females with an annual incidence rate of approximately 3.5 per million for males and 2.9 per million for females under the age of 24 years [3].

The etiology of osteosarcoma is largely unknown [1, 4]. In humans, ionizing radiation is the only environmental agent known to cause bone cancer and is thought to have an effect in approximately 3% of cases from either external high-dose irradiation used in cancer therapy or internal bone-seeking radioisotopes from occupational or medical use [1, 5, 6]. Alkylating agents used in chemotherapy are thought to increase the risk for osteosarcoma and evidence for other etiologic factors including viruses, antecedent trauma, or radium in drinking water has been suggested but inconclusive [1, 5, 7, 8]. Certain pre-existing bone defects including Paget's disease have been found more frequently in patients who subsequently developed bone cancers [1, 4, 5]. Also, a genetic predisposition for osteosarcoma has been described, specifically for patients with a hereditary form of retinoblastoma or those with familial Li-Fraumeni cancer syndrome [1, 5, 6].

The age-incidence distribution of osteosarcoma is bimodal, raising the possibility of different risk factors contributing to the incidence of osteosarcoma at different ages. The first and larger peak in incidence occurs in the second decade of life and a subsequent peak occurs in males in the eighth decade of life [2, 4, 5, 9]. Evidence suggests that osteosarcoma is associated with skeletal growth, particularly for patients diagnosed during adolescence [1, 9–11]. Since fluoride may act as a mitogen (increasing the proliferation of osteoblasts) and its uptake in bone increases when skeletal growth is more rapid [12, 13], it is biologically plausible that fluoride exposure during growth is associated with the subsequent development of osteosarcoma, and fluoride could either increase or decrease the rate of osteosarcoma.

There are conflicting data regarding the association between fluoride exposure and the incidence of osteosarcoma. Several animal studies have been conducted, but only one found evidence that fluoride exposure may increase osteosarcoma formation, specifically in male rats [14]. Human studies also show conflicting results. The majority of epidemiologic studies found no association between fluoride and osteosarcoma [15–21]. However, two studies found evidence of an association in males under age 20, but not in females [22, 23]. Furthermore, prior studies have primarily evaluated fluoride exposure at the time of diagnosis or as an average lifetime exposure and have not evaluated exposure at specific ages during growth and development when cell division is occurring rapidly.

Therefore, we use data from the Harvard Fluoride Osteosarcoma Study [24] to explore age-specific and sex-specific effects and evaluate exposure to fluoride in drinking water from birth through early adolescence. Specifically, based on prior studies suggesting an effect of fluoride limited to males under age 20, we limited our analysis to the first two decades of life and evaluated effects in males and females separately.

Materials and methods

We used data from a hospital-based, matched case–control study which evaluated lifetime exposure to fluoride from drinking water and self-administered fluoride products [24]. Subjects were identified through the orthopedic departments at 11 teaching hospitals across the United States. Cases had histologically confirmed osteosarcoma diagnosed between November 1989 and November 1992. Exclusion criteria were: age 40 years or older, any history of radiation therapy or a history of renal dialysis. Controls were patients of the same hospital's orthopedics department, seen within ±6 months of the case's diagnosis and matched with cases on age (±5 years), gender, distance from hospital, with the same exclusion criteria applied to cases. Telephone interviews were conducted between January 1992 and January 1995 with the parent or subject (over 18 years old) or with a proxy if subjects were deceased or incapacitated. Interviewers collected information which included a complete residential history, use of fluoride supplements and mouth rinses. Study procedures were approved by the Harvard Medical School Committee on Human Studies and each of the participating institutions. Data on a total of 419 subjects, 139 cases and their 280 matched controls, were available based on eligibility criteria, matching criteria and a completed interview. However, we limit the current analysis to 103 cases less than 20 years old and 215 controls matched to these cases.

Fluoride level in drinking water was the primary exposure of interest. The interview obtained the usual type of the subject's drinking water (municipal, private well, bottled) and the subject's age(s) while at each address. From these data, we estimated the level of fluoride in drinking water for each subject at each age, and explored the effects of fluoride during their growth and development. To estimate fluoride concentration for public water supplies, we obtained preliminary data from the 1985 CDC Fluoridation Census [25] and the 1992 CDC Fluoridation Census [26]. We then contacted state agencies (State Dental Director's Office, State EPA Office of Drinking Water, Water Administrators Office) and local sources (county health departments, the town or city clerk's office and specific water systems) to confirm and supplement the CDC data [27]. For subjects who drank well water, a sample was obtained from current or former residents for the specific appropriate addresses. Fluoride concentrations were measured at Harvard School of Dental Medicine using a Colorimeter (Model 41100-21, Hach Company, Ames, IA). Subjects who used bottled water as their usual source of drinking water were identified, but information about specific brand was not collected. We estimated fluoride

levels to be 0.1 parts per million (ppm) in bottled water based on the weighted average of fluoride concentration in leading brands [28]. Since subjects who used bottled water were also likely to consume fluoride from tap water in food and beverage preparation and use outside the home (e.g., school), we used the mean of fluoride estimates for bottled water (i.e., 0.1 ppm) and municipal water for these residences. Since water consumption may vary based on climate, we standardized fluoride exposure estimates based on CDC recommendations for optimal target levels of fluoride [29]. For example, for locations in warmer climates where the target fluoride level is 0.7 ppm, we divided fluoride levels by 0.7, while for locations in colder climates where the target fluoride level is 1.2 ppm we divided by 1.2. The standardization of fluoride exposure was done for all three types of drinking water.

We created a proxy measure for socioeconomic status (SES) by linking zip code at the time of diagnosis with data from the Census Bureau that provide 1989 median family income for each zip code. Median family income was categorized into quartiles based on the distribution for controls. We also used data from the Census Bureau to determine the 1990 population of the county where subjects resided at the time of diagnosis, categorized by approximate tertiles. We examined type of drinking water by including indicators for use of bottled water or well water at any time up to the exposure age. Since age matching allowed for a difference as large as 5 years, we included age (at diagnosis for cases and at time of hospital treatment for controls) as a covariate. Lastly, since information was collected for use of self-administered fluoride products at home or in school-based programs, we included an indicator for any use of these products as an additional covariate.

We used conditional logistic regression to estimate the odds ratio for the association between fluoride exposure and osteosarcoma, taking into account the matching between cases and controls. The dependent variable was an indicator identifying cases and the primary independent variables were measures of fluoride exposure. We fit two basic models. The first model included only the exposure measures as independent variables. The second model also included age, a proxy for SES, county population, use of private well water or bottled water, and any use of fluoride supplements or mouth rinses as covariates. In this analysis, our a priori hypothesis was that fluoride exposure may have sex-specific differential effects on osteosarcoma risk based on age at exposure. The models we employed therefore do not assess the question of average induction time or latency.

We report the mean and standard deviation of fluoride levels in ppm and percent of target for each specific age. To examine the association between osteosarcoma and fluoride exposure at specific ages, we fit separate models for each exposure age up to the age of diagnosis for each case and the same age for the matched controls. Each model included the age-specific fluoride level and a sex–fluoride interaction term. In this analysis we expect substantial correlation in exposure to fluoride in drinking water from year to year, limiting our ability to identify age-specific effects precisely. For our primary analysis we categorized climate-standardized fluoride exposure into three categories ($<30\%$, 30–99%, $>99\%$ of target fluoride content) corresponding to approximate tertiles based on the distribution among controls. We plot sex-specific estimates of the odds ratio and 95% confidence intervals as a function of exposure age. We also fit a model using fluoride exposure categorized without standardization by climate into three groups (<0.3, 0.3–0.69, and ≥ 0.7 ppm).

We performed a sensitivity analysis on our assumption that the fluoride content of bottled water is 0.1 ppm by fitting models using values as high as 0.5 ppm for bottled water (assuming that bottled water and municipal water each contributed half of the consumption for subjects who used bottled water). In addition, we conducted a sensitivity analysis evaluating the age-specific and sex-specific effects of fluoride in drinking water among subjects who reported never having used any fluoride supplements or fluoride mouth rinses.

Results

A total of 157 cases diagnosed before age 20 were identified at the participating hospitals. No interviews were completed for 13 of the cases (did not attempt to contact, could not contact, or respondent refused). Eleven cases used well water for which no sample was obtained and 12 cases lived outside the United States for more than 6 months. An additional 18 cases with interview data were excluded due to lack of appropriately matched controls (nine had no eligible matches identified or successfully interviewed, seven whose only matches used well water for which no sample was obtained and two whose matches lived outside the United States >6 months). Characteristics of the remaining 103 cases and their 215 matched controls are presented in Table 1. Cases were diagnosed at a median age of 14 years (range 6–19, interquartile range 11–17). Residential histories for six participants, five cases and one control, were provided by proxies (grandparents, stepparent, sibling, aunt, neighbor). The 1989 median family income for zip code of residence was lower for cases than controls (P=0.01, Student's t-test) and a larger proportion of controls used bottled water (P=0.002, chi-square test). Table 2 shows the average fluoride level and percent of climate-specific target level in drinking water at each age for cases and controls.

Table 1 Characteristics of study population[a]

	Cases	Controls
Number	103	215
Age (years)	13.7 ± 3.5	14.5 ± 3.9
Gender		
Male	60 (58%)	122 (57%)
Female	43 (42%)	93 (43%)
Self-reported race[b]		
White	81 (79%)	180 (84%)
Black	16 (16%)	23 (11%)
Asian	3 (3%)	2 (1%)
Other	3 (3%)	9 (4%)
Number of residences	2.5 ± 1.7	2.6 ± 1.7
1989 Median family income[c]	$41,458 ± 15,146	$46,841 ± 19,319
County population[c]		
<250,000	37 (37%)	69 (32%)
250,000–999,999	44 (44%)	86 (40%)
1,000,000+	19 (19%)	60 (28%)
Hospital		
MGH	17 (17%)	27 (13%)
CH, Boston	15 (15%)	45 (21%)
Creighton	5 (5%)	11 (5%)
CH, DC	11 (11%)	20 (9%)
MSKCC	7 (7%)	14 (7%)
U Chicago	8 (8%)	16 (7%)
Rush	3 (3%)	6 (3%)
U Florida	12 (12%)	19 (9%)
UCLA	14 (14%)	32 (15%)
Cleveland clinic	8 (8%)	19 (9%)
CWRU	3 (3%)	6 (3%)
Ever well water use	29 (28%)	44 (20%)
Ever bottled water use	8 (8%)	46 (21%)
Fluoride Products		
Rinses	3 (3%)	19 (9%)
School program	17 (17%)	30 (14%)
Tablets	10 (10%)	28 (13%)
Drops	9 (9%)	19 (9%)
Any of above	27 (26%)	77 (36%)

[a] Values reported are mean ± standard deviation or *n* (%)

[b] Race not available for one control

[c] 1989 Median family income and county population data not available for three cases

Figure 1 shows the odds ratio, relative to the lowest exposure group, of osteosarcoma for the climate-standardized fluoride level at each exposure age from 0 to 14 years, estimated using the conditional logistic regression models unadjusted for other covariates. Among males, exposure to fluoride at or above the target level was associated with an increased risk of developing osteosarcoma (Fig. 1a). The association was most apparent between ages 4 and 12 with a peak at 6–8 years of age. The odds ratio for the high exposure group was 4.07 at 7 years of age with a 95% confidence interval of 1.43–11.56. Among females less than 20 years old, no association between fluoride in drinking water and osteosarcoma was apparent at any age (Fig. 1b).

Next we fit models with all the covariates. As an example, Table 3 shows the model for subjects at 7 years

Table 2 Fluoride level for drinking water[a]

	F level in ppm		Percent of target	
	Cases	Controls	Cases	Controls
Age (years)				
0	0.63 ± 0.40	0.60 ± 0.41	66% ± 41%	62% ± 41%
1	0.63 ± 0.40	0.60 ± 0.40	65% ± 41%	61% ± 40%
2	0.64 ± 0.40	0.61 ± 0.40	67% ± 41%	63% ± 40%
3	0.67 ± 0.39	0.63 ± 0.39	69% ± 40%	64% ± 39%
4	0.70 ± 0.40	0.62 ± 0.39	73% ± 41%	63% ± 39%
5	0.69 ± 0.40	0.63 ± 0.39	72% ± 41%	65% ± 38%
6	0.70 ± 0.40	0.62 ± 0.39	74% ± 41%	63% ± 39%
7	0.70 ± 0.38	0.61 ± 0.39	75% ± 40%	63% ± 39%
8	0.69 ± 0.38	0.61 ± 0.39	73% ± 40%	63% ± 38%
9	0.68 ± 0.39	0.63 ± 0.38	73% ± 41%	65% ± 38%
10	0.67 ± 0.39	0.61 ± 0.39	71% ± 41%	63% ± 39%
11	0.70 ± 0.56	0.60 ± 0.39	74% ± 65%	62% ± 39%
12	0.69 ± 0.56	0.59 ± 0.39	75% ± 66%	61% ± 39%
13	0.68 ± 0.39	0.61 ± 0.39	71% ± 41%	62% ± 38%
14	0.65 ± 0.41	0.59 ± 0.39	69% ± 43%	61% ± 38%

[a] When bottled water was used, the estimate was 0.1 ppm for bottled water and it was assumed that bottled water and municipal supply each accounted for 50% of consumption

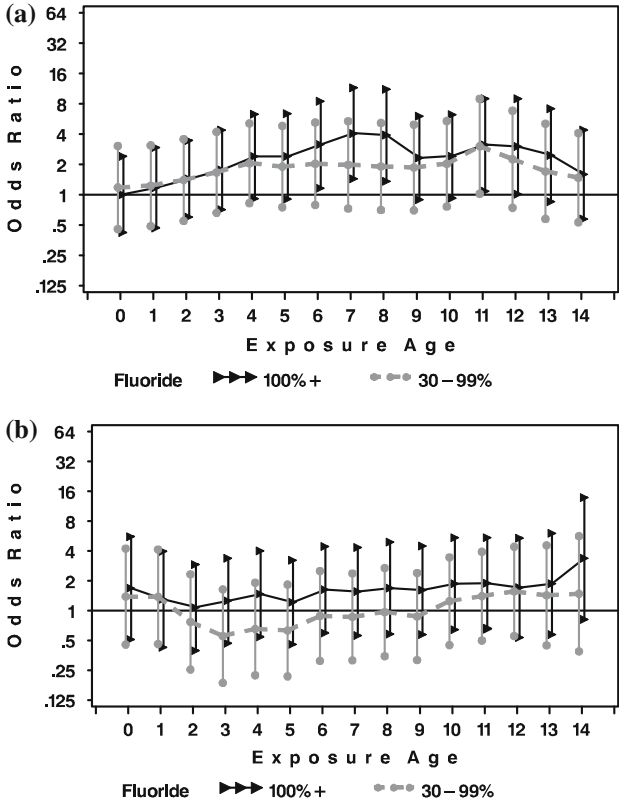

Fig. 1 Odds ratios and 95% confidence intervals relative to fluoride levels less than 30% of target are shown for males (panel a) and for females (panel b). The dashed line shows the odds ratios for the intermediate exposure category (30–99% of target fluoride level) and the solid line shows the odds ratios for the high exposure category (100% of target or greater)

Table 3 Sex-specific associations between fluoride exposure at age 7 years and osteosarcoma, estimated by conditional logistic regression

Fluoride exposure at age 7 years	Odds ratio (95% C.I.)[a]
Males	
Less than 30% of target	1.00
30–99% of target	3.36 (0.99, 11.42)
At least 100% of target	5.46 (1.50, 19.90)
Females	
Less than 30% of target	1.00
30–99% of target	1.39 (0.41, 4.76)
At least 100% of target	1.75 (0.48, 6.35)

[a] Adjusted for age, zip code median income, county population, use of well water by age 7, use of bottled water by age 7, any use of fluoride supplements

of age. Figure 2 shows a similar effect of fluoride level in drinking water after adjusting for income by zip code, county population, ever use of bottled or well water, age, and any use of self-administered fluoride products. For males, the odds ratio for the high exposure group was 5.46 at 7 years of age with a 95% confidence interval of 1.50–19.90. Sensitivity analyses, which assumed that the fluoride

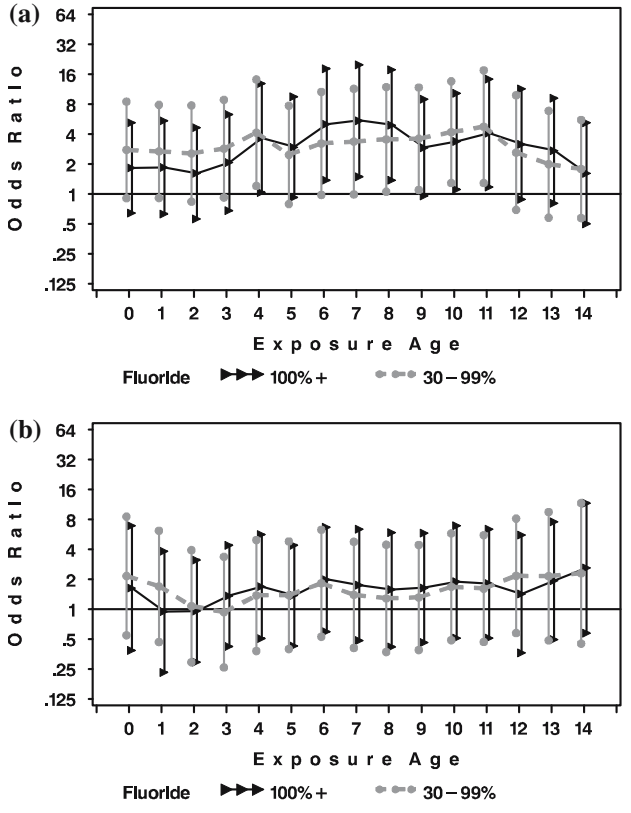

Fig. 2 Odds ratios and 95% confidence intervals relative to fluoride levels less than 30% of target are shown for males (panel a) and for females (panel b). The dashed line shows the odds ratios for the intermediate exposure category (30–99% of target fluoride level) and the solid line shows the odds ratios for the high exposure category (100% of target or greater). Estimates are adjusted for age, zip code median income, county population, prior use of well water, prior use of bottled water, and any use of fluoride supplements

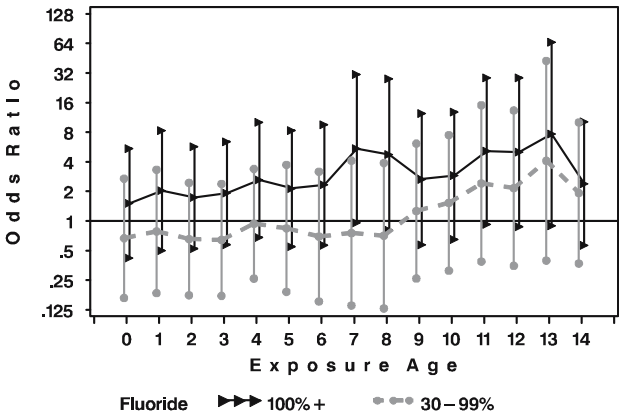

Fig. 3 Odds ratios and 95% confidence intervals relative to fluoride levels less than 30% of target are shown for the subset of male participants who never used fluoride supplements or rinses. The dashed line shows the odds ratios for the intermediate exposure category (30–99% of target fluoride level) and the solid line shows the odds ratios for the high exposure category (100% of target or greater)

content of bottled was as low as 0.1 ppm or as high as 0.5 ppm, yielded essentially identical results. A sensitivity analysis that categorized fluoride exposure based on the absolute fluoride concentration, without standardizing for climate-specific target fluoride level, also showed essentially the same results (unadjusted OR=3.77; 95% CI 1.41, 10.05, and adjusted OR=5.55; 95% CI 1.60, 19.24 for 0.7 ppm or greater relative to less than 0.3 ppm). To avoid potential confounding by fluoride supplementation or fluoride rinses, we conducted a sensitivity analysis restricting our population to subjects who reported that they did not use supplements or rinses. This substantially reduced the sample size limiting us to unadjusted analyses for males. The results were consistent (Fig. 3).

Discussion

Our exploratory analysis described the association of fluoride level in drinking water at specific ages and the incidence of osteosarcoma. We observed that for males diagnosed before the age of 20 years, fluoride level in drinking water during growth was associated with an increased risk of osteosarcoma, demonstrating a peak in the odds ratios from 6 to 8 years of age. All of our models were remarkably robust in showing this effect, which coincides with the mid-childhood growth spurt [30–33]. For females, no clear association between fluoride in drinking water during growth and osteosarcoma emerged.

We found similar effect magnitudes in the intermediate and high exposure levels, as opposed to a dose–response gradient. This may be due to misclassification of the primary exposure for some artificially fluoridated systems.

Reeves [34] reported that only 65% of fluoridated water systems routinely have target levels of fluoride maintained in the drinking water, which may result in our misclassifying up to 35% of the adjusted water systems, categorizing them in the highest group (100% of target or greater) when some truly belong in the middle group (30–99% of target). While non-differential misclassification of exposure results in bias towards the null for a dichotomous exposure, Birkett [35] has shown that with three levels of exposure, the estimated odds ratio for the highest exposure level is biased towards the null, but for the intermediate category the estimate can be biased in either direction. Hence, in our study the misclassification might mask an effect that increases with dose.

Our results are consistent with a pattern seen in the National Toxicology Program (NTP) animal study and two ecological studies. The NTP animal study, which reported ''equivocal evidence'' for an association between fluoride and osteosarcoma, found a positive association for male rats, but no association for female rats or mice of either gender [14]. Using data from the Surveillance, Epidemiology and End Results (SEER), Hoover et al. found an unexplained increase in osteosarcoma in males less than 20 years of age in fluoridated versus non-fluoridated areas. However, a time-trend analysis which took into account the duration of fluoride exposure failed to demonstrate a higher incidence among males exposed to fluoridated water their entire lives than among those exposed less than half their lives [22]. A similar, but smaller study examining osteosarcoma in New Jersey also showed an increase in incidence rates for males less than 20 years old who lived in fluoridated areas compared to those living in non-fluoridated areas [23].

A number of other case–control studies did not find an association between fluoride in drinking water and osteosarcoma [18–21]. In addition, preliminary analyses of an ongoing case–control study of the determinants of osteosarcoma conducted at the same network of hospitals that participated in the present study and recruited cases during their initial hospitalization, found no overall association between lifetime exposure to fluoride or fluoride content in bone biopsies, a marker of cumulative exposure, and osteosarcoma (personal communication, Chester Douglass, D.M.D., Ph.D.). This lack of agreement may be related to the bimodal age-incidence distribution of osteosarcoma [2, 4, 5, 9]. When there are two distinct peaks in an age-incidence distribution, two distinct sets of component causes should be considered [36]. McGuire et al. [19] and Moss et al. [20] included cases up to age 40 years and 84 years, respectively, and if fluoride exhibits a different effect according to the age-specific distribution, detecting an effect would be unlikely. Operskalski et al. [18] selected friends and neighbors of the cases as controls, which might have been optimal for some exposures of interest, but resulted in inadvertently matching on drinking water fluoride level. The evaluation of age-specific effects distinguishes our study from the other investigations. Rothman [37] has warned that failure to identify the appropriate time window for exposure may result in misclassification which can adversely affect the ability to detect an association. This might explain why the study by Gelberg et al. [21] did not find an association between fluoride in drinking water and osteosarcoma since age-specific effects were not evaluated.

It is biologically plausible that fluoride affects the incidence rate of osteosarcoma, and that this effect would be strongest during periods of growth, particularly in males. First, approximately 99% of fluoride in the human body is contained in the skeleton with about 50% of the daily ingested fluoride being deposited directly into calcified tissue (bone or dentition) [13]. Second, fluoride acts as a mitogen, increasing the proliferation of osteoblasts [12, 38] and its uptake in bone increases during periods of rapid skeletal growth [13]. In the young, the hydroxyapatite structure of bone mineral exists as many extremely small crystals each surrounded by an ion-rich hydration shell, providing a greater surface area for fluoride exchange to occur [39, 13]. Also, osteosarcoma, for the ages we considered, generally originates in the metaphyseal areas of long bones [2] and the pattern of the blood supply to the metaphyses and epiphyses, where growth of long bones takes place, differs from that of the diaphyses because of the special circulation to the epiphyseal growth plate in the young which in turn disappears when growth is complete [40, 41]. Lastly, the amount of fluoride present in bone depends on gender and intake [39] and intake, on average, is greater for males than females for all ages over 1 year [42].

There are several limitations to our study. First, our estimates of fluoride in drinking water at each residence do not reflect actual consumption by subjects and the study did not obtain biologic markers for fluoride uptake in bone. However, dietary sources of fluoride comprise the majority of human exposure [13], and for individuals living in fluoridated communities, the fluoride in drinking water is estimated to contribute two-thirds of the total dietary intake [39]. Also, when we added use of self-administered (home- or school-based) products as a covariate in the model, there was no substantial change in results. The *halo* or *diffusion* effect, described in the dental literature, refers to people in non-fluoridated communities receiving fluoride from food and beverages processed in fluoridated communities and vice versa [43]. We would expect this type of measurement error to result in a bias towards underestimating any true effect that might exist.

Because cases and controls moved rarely up to the age at diagnosis (an average of 1.5 times) leading to essentially

collinear exposure from year to year, we were unable to apply statistical models that assess the effect of age-specific exposure while simultaneously adjusting for exposure at other ages such as distributed lag models. Residential histories were obtained from proxies more often among cases than controls, however the absolute number was small and the proxies were generally close relatives.

The estimation of fluoride concentration at each residence is subject to several sources of measurement error. Monitoring guidelines for fluoridated water systems permit actual fluoride levels to vary. For example, if the target fluoride concentration for a specific water system is 1.0 ppm, guidelines may consider values between 0.8 and 1.3 ppm acceptable. Also, natural fluoride levels may vary over time, but they are unlikely to do so for the length of time subjects lived at their respective address unless the water source changed. For bottled-water users, we did not know the specific brands consumed and a small proportion of brands on the market do have substantial levels of fluoride. However, analysis of the leading national brands makes a value of 0.1 ppm a reasonable estimate [28]. Further, we demonstrated that our findings were not sensitive to this assumption.

The lack of data available for other potential confounders is also a limitation. Fluoride may not be the causative agent; instead there may be another factor in drinking water correlated with the presence of fluoride. Data to assess fluoride exposure in diet, industrial fluoride exposure or other fluoride exposures (e.g., pesticides) were not available. Instead, by including type of drinking water subjects used (ever well, ever bottled) as a covariate, we may have partially controlled for some of the ''other unknown factors'' such as contaminants or carcinogens subjects might have been exposed to irrespective of fluoride concentration in these natural sources or products.

Another limitation is the possibility of selection bias. In our case–control study, the secondary study base is defined by the cases and in order for the results to be valid the exposure distribution for controls must represent the exposure distribution in this theoretical population. Referral patterns to the participating hospitals may differ for cases and controls because the participating hospitals were primary referral centers for osteosarcoma for large regions but the controls likely represented a more proximate population. Further, for some of the hospitals the referral base for controls could represent different socioeconomic populations than for cases. Distance from hospitals was used as a matching factor, to limit selection bias. This matching factor could also result in some overmatching on exposure, resulting in possible underestimation of the effect. Additionally, we included the 1989 median family income and county population as covariates.

For this study, cases of osteosarcoma that were diagnosed at participating hospitals between November 1989 and November 1992 were identified. However, case and control interviews took place later, between January 1992 and January 1995. Although efforts were made to interview a parent or proxy respondent if the subject was deceased or incapacitated, it is possible that cases with more favorable prognosis may have been over-sampled. If this occurred, an alternative explanation for our observation is that boys exposed to higher levels of fluoride who subsequently develop osteosarcoma have a better prognosis than boys exposed to lower levels. While we cannot rule out this possibility, the magnitude of the protective effect that would be required to explain the observed association is unlikely.

Differential recall of exposure information between cases and controls is unlikely in the current study because respondents did not provide information about the fluoride level in their drinking water but rather a complete residential history. For other covariates, such as date of birth, sex, or zip code at time of diagnosis, information was obtained by medical record review. Reporting of the type of water used or the use of self-administered fluoride products could be affected by recall bias.

In summary, this exploratory analysis found an association between exposure to fluoride in drinking water and the incidence of osteosarcoma, demonstrating a peak in the odds ratio for exposure at ages 6–8 years among males diagnosed less than 20 years old, but no consistent association among females. Future studies would benefit from the inclusion of biomarkers of fluoride exposure and assessment of potential gene–environment interactions. Such studies with larger numbers of osteosarcoma patients, with incidence under age 20, that examine age-specific and sex-specific associations are required to confirm or refute the findings of the current study.

Acknowledgements The authors acknowledge Manuela Orjuela, MD, ScM, for her contributions on a preliminary analysis. This work was supported by the National Institutes of Health Grant number 5-R01 ES06000. Dr Bassin was supported by National Research Service Award grant T32 DE07151-13.

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