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Tewodros Rango
Duke University, tg67@duke.edu

Avner Vengosh
Duke University

Marc Jeuland
National University of Singapore

Redda Tekle-Haimanot
Addis Ababa University

Erika Weinthal
Duke University

See next page for additional authors

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Authors
Tewodros Rango, Avner Vengosh, Marc Jeuland, Redda Tekle-Haimanot, Erika Weinthal, Julia Kravchenko, Christopher Paul, and Paul McCornick

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Fluoride Exposure from Groundwater as Reflected by Urinary Fluoride and Children's Dental Fluorosis in the Main Ethiopian Rift Valley

Tewodros Rango,[1] Avner Vengosh,[1] Marc Jeuland,[2,7]

Redda Tekle-Haimanot,[3] Erika Weinthal,[4] Julia Kravchenko,[5]

Christopher Paul,[4] and Peter McCornick[6]

1. Division of Earth and Ocean Sciences, Nicholas School of the Environment, Duke University, Durham, North Carolina, USA
2. Sanford School of Public Policy and Duke Global Health Institute, Duke University, Durham, North Carolina, USA
3. Addis Ababa University, Faculty of Medicine, Addis Ababa, Ethiopia
4. Division of Environmental Sciences and Policy, Nicholas School of the Environment, Duke University, Durham, North Carolina, USA
5. Duke University Medical Center, Department of Surgery, Division of Surgical Science, Duke University, Durham, North Carolina, USA
6. International Water Management Institute, Colombo, Sri Lanka
7. Institute of Water Policy, National University of Singapore, Singapore

Corresponding author – T. Rango, telephone +1-919-949-8783, email tg67@duke.edu

Abstract

This cross-sectional study explores the relationships between children's F^- exposure from drinking groundwater and urinary F^- concentrations, combined with dental fluorosis (DF) in the Main Ethiopian Rift (MER) Valley. We examined the DF prevalence and severity among 491 children (10 to 15 years old) who are lifelong residents of 33 rural communities in which groundwater concentrations of F^- cover a wide range. A subset of 156 children was selected for urinary F^- measurements. Our results showed that the mean F^- concentrations in groundwater were 8.5 ± 4.1 mg/L (range: 1.1–18 mg/L), while those in urine were 12.1 ± 7.3 mg/L (range: 1.1–39.8 mg/L). The prevalence of mild, moderate, and severe DF in children's teeth was 17%, 29%, and 45%, respectively, and the majority (90%; n = 140) of the children had urinary F^- concentrations above 3 mg/L. Below this level most of the teeth showed mild forms of DF. The exposure-response relationship between F^- and DF was positive and nonlinear, with DF severity tending to level off above a F^- threshold of ~6 mg/L, most likely due to the fact that at ~6 mg/L the enamel is damaged as much as it can be clinically observed in most children. We also observed differential prevalence (and severity) of DF and urinary concentration across children exposed to similar F^- concentrations in water, which highlights the importance of individual-specific factors in addition to the F^- levels in drinking water. Finally, we investigated urinary F^- in children from communities where defluoridation remediation was taking place. The lower F^- concentration measured in urine of this population demonstrates the capacity of the urinary F^- method as an effective monitoring and evaluation tool for assessing the outcome of successful F^- mitigation strategy in a relatively short time (months) in areas affected with severe fluorosis.

Keywords: drinking water quality, urinary biomarker, exposure-response, defluoridation, risk assessment, East Africa

1. Introduction

Globally, an estimated 200 million people are exposed to high concentrations of naturally occurring fluoride (F^-) that exceeds the World Health Organization (WHO) guideline of 1.5 mg/L in drinking water (Ayoob and Gupta, 2006; WHO, 2006). This high exposure to F^- leads to fluorosis—in its dental and skeletal forms—and is endemic in at least 25 countries, including India, China, Mexico, Brazil, Saudi Arabia, United States (U.S.), Uganda, Tanzania, and Ethiopia (WHO, 2006; Amini et al., 2008). High-risk areas are mostly located in arid and semiarid regions that are characterized by a rapid rate of chemical weathering of geological materials, such as the East African Rift System (EARS).

The EARS is a unique geological feature where active faulting has generated voluminous pyroclastic volcanic rocks (Chorowicz, 2005) that are highly reactive with local groundwater (Rango et al., 2013). This study focuses on the Main Ethiopian Rift (MER), which is located in the northern part of EARS, and where a large number of drinking water wells have been documented to contain high levels of naturally occurring contaminants such as F^-, arsenic (As), and uranium (U) (Reimann et al., 2003; Rango et al., 2012, 2013). Systematic water testing in the Ziway-Shala basin of the MER has shown that F^- concentrations can reach up to 68 mg/L (mean: 9.4 ± 10.5 mg/L), and that F^- levels in 94% of the tested wells exceeded the World Health Organization (WHO) standard of 1.5 mg/L (Rango et al., 2012). In this region, an estimated 8.5 million people, mostly from rural communities, are highly dependent on groundwater resources for drinking and domestic purposes and are thus at risk of fluorosis (Tekle-Haimanot et al., 1987; Tekle-Haimanot, 2005; Tekle-Haimanot and Haile, 2014).

Exposure to F^- has two critical effects on the teeth. On the one hand, optimum intake of this element is critical for dental development; F^- intake of 0.5–1 mg/L is recommended to achieve maximum protection against dental caries (U.S. DHHS, 1991; WHO, 2006). Indeed, fluoridation of community drinking water is considered a safe and effective means of preventing such caries and has been called one of the ten great public health achievements of the 20th century (U.S. CDC, 1999). On the other hand, excessive intake of F^- from sources such as water, food, and fluoride-containing dental products is known to cause dental and skeletal fluorosis (DF and SF) (WHO, 2006). DF—the focus of this study—is a condition of subsurface enamel porosity that may progress to enamel pitting, followed by total enamel loss and secondary discoloration of the enamel surface (Fejerskov et al., 1996).

The severity of DF depends on the complex interplay of exposure, duration, and timing of F^- intake and ingestion (Den Besten, 1994). It is particularly acute when children are exposed to high levels of F^- in early childhood (typically at ages up to 4 years) (Fomon et al., 2000; U.S. CDC, 2001; Hong et al., 2006). To achieve dental protection without compromising health, the U.S. Environmental Protection Agency (EPA) has thus specified the optimal level of 0.06 mg/kg bw/day as the No-Observed-Adverse-Effects-Level (NOAEL) (U.S. EPA, 2002). The NOAEL is an estimate of the daily F^- exposure that does not lead to cosmetic DF effects (brown staining and/or pitting of enamel) among children. For a F^- intake from drinking water through the consumption of 1 L/day by 12- to 14-year-old children, the NOAEL corresponds to a concentration of about 1 mg/L of F^- (U.S. EPA, 2002). The WHO guideline for drinking water is 1.5 mg/L, but the guidelines note that when water intakes are high, for example in arid and semiarid settings, it may be appropriate to consider a local guideline concentration that is lower than 1.5 mg/L (WHO, 2006).

It is indisputable that F^- in drinking water is the primary factor that causes DF; however, the precise exposure-response condition has not been well established, in part because of the difficulty of tracking varying exposures over long and critical periods of dental development. Previous studies, for example in the United States, have demonstrated a linear dose-response relationship at low-F^- intakes, i.e., mostly below 4 mg/L from drinking water (U.S. NRC, 2006). Very few studies—e.g., Ruiz-Payan et al. (2005) (covering water sources < 5.7 mg/L in Mexico), Wang et al. (2012) (< 11 mg/L, mostly below 7 mg/L in China), and Wondwossen et al. (2004) (including low (0.3–2.2 mg/L) and high F^- (10–14 mg/L) concentrations of F^- in the Ethiopian Rift Valley)—have considered the development of DF across a wide range of F^- exposures in a specific geographic region. There are also challenges related to confounding by other sources of exposure: for example, existing studies from the MER have shown that food ingredients and food or beverages prepared with high F^- water contribute significantly to total F^- intake (Malde et al., 1997, 2003, 2004, 2011; Dessalegne and Zewege, 2013). Based on the available research evidence, the U.S. EPA established a MCLG (Maximum-Contaminant-Level Goal) threshold of 4 mg/L to protect from adverse health effects (crippling skeletal fluorosis) and a SMCL (Secondary-Maximum-Contaminant-Level) threshold of 2mg/L of F^- to protect from adverse cosmetic effects (moderate and/or severe DF) (U.S. NRC, 2006). Yet it is not clear whether the F^- exposure thresholds established by the U.S. EPA, or by the WHO, are valid or applicable in other countries with different climates, exposure sources and pathways, and population characteristics, such as those in Ethiopia.

In this paper, we describe the results of an exposure-response study of the effects of F^- that was conducted in the MER. The study builds on prior work in the same region that considered the relationship between F^- in groundwater and DF (Rango et al., 2012) by more carefully: (1) specifying the full range of F^- concentrations in groundwater encountered in this region; (2) restricting the sample to the specific age range (10 and 15 years) of children; (3) limiting threats related to confounding by including only individuals who are lifelong residents of rural communities in which the primary community drinking water supplies were installed before the children were born; and (4) generating new data on urinary F^- concentration and establishing their relationship with exposures to F^- in groundwater and DF severity. Because of the temporal stability and spatial variability in F^- levels across communities (ranging from 1.1 to 18 mg/L) in these sources the study of this population provides us a unique opportunity to make inferences about the relationship between exposure and health effects over a wide range of F^- concentrations. Working with this population, we investigated whether there might be thresholds for drinking water F^- concentrations for either minimal or severe DF.

Our study contributes to a relatively limited literature that examines the relationship between F^- levels measured in drinking water and urine among a subset of study subjects and is one of the only ones to consider such a wide range of F^- exposures. In the human body, approximately 99% of the F^- is stored in calcified tissues (i.e., bones and teeth) (Whitford, 1996). Roughly 30–50% of the F^- absorbed every day by young to middle-aged adults is assimilated within 24 h by calcified tissues as compared to about 80% by young children, and the remainder is predominantly excreted in the urine (Ekstrand et al., 1994; Whitford, 1996). Prolonged exposure to steady and high concentrations of F^- can yield urinary F^- excretion above 80% of the total F^- intake, particularly when mineralized tissues are close to saturation with F^- (Myers, 1978). Based on this premise, we supplemented analyses of drinking water and DF examinations with measures of urinary F^- concentrations, in order to more adequately monitor recent F^- exposure (Whitford, 1994; Singh et al., 2007; Srikanth et al., 2013). We also evaluated urinary F^- concentrations in a community with an active pilot defluoridation intervention to provide an initial understanding of the short-term effect of defluoridation on this biomarker. To date, studies have largely been conducted in areas with either exclusively low (e.g., Czarnowski et al., 1996 (< 1.2 mg/L); Heintze et al., 1998 (< 1.3 mg/L); Villa et al., 2000 (< 0.6 mg/L); Forte et al., 2008 (< 1.5 mg/L); Zohouri and Rugg-gunn, 2000 (< 0.4 mg/L); Ding et al., 2011 (< 3 mg/L); Zohouri et al., 2013 (< 1.06 mg/L)) or high F^- in drinking water (e.g., Ruiz-Payan et al., 2005 (up to 5.7 mg/L); Wang et al., 2012 (mostly below 7 mg/L)).

The present study thus provides more comprehensive evidence on the effects of a wide range of exposures to F^- on DF than the majority of existing studies. The study population from the MER was found to be an ideal research group for these exposure-response investigations because of the relative homogeneity of the population being studied (in terms of diet, ethnicity, and rural location), its high reliance on specific groundwater sources for drinking water in which concentrations of F^- are temporally stable, and the low potential for confounding given the limited ingestion of other products containing F^-, such as industrial (e.g., processed diet and soft drinks) or topical (e.g., toothpaste) products. Finally, our analyses also consider the role of potential modifiers (such as sex, age, nutritional status, and breastfeeding history) to DF outcomes.

2. Materials and methods

2.1. Field measurements

2.1.1. Measurement of F⁻ in groundwater

Groundwater samples were collected from 94 community wells during the dry season (April–May 2010, March 2011, and November 2012) (Fig. 1). The samples were collected from active pumping wells that were primarily used for drinking water. Water was allowed to flow for a few minutes from wells prior to sampling. The F^- concentration in groundwater was measured in-situ and determined electrochemically using the Thermo Scientific Orion Ion-Selective Electrode (ISE) (results were confirmed using the Ion Chromatography method) following a procedure reported by Singh et al. (2007) and Ruiz-Payan et al. (2005). The water samples were diluted with equal volume ratio with a total ionic strength adjustment buffer (TISAB II) of pH 5–5.5, which allows for optimal analyses of F^- in aqueous solution. Calibration standards were prepared from 100 mg/L stock solution. The mean electrode calibration slope for a 10-fold change in F^- concentration was −58.4 ± 0.6 mV, which is within acceptable theoretical slope range.

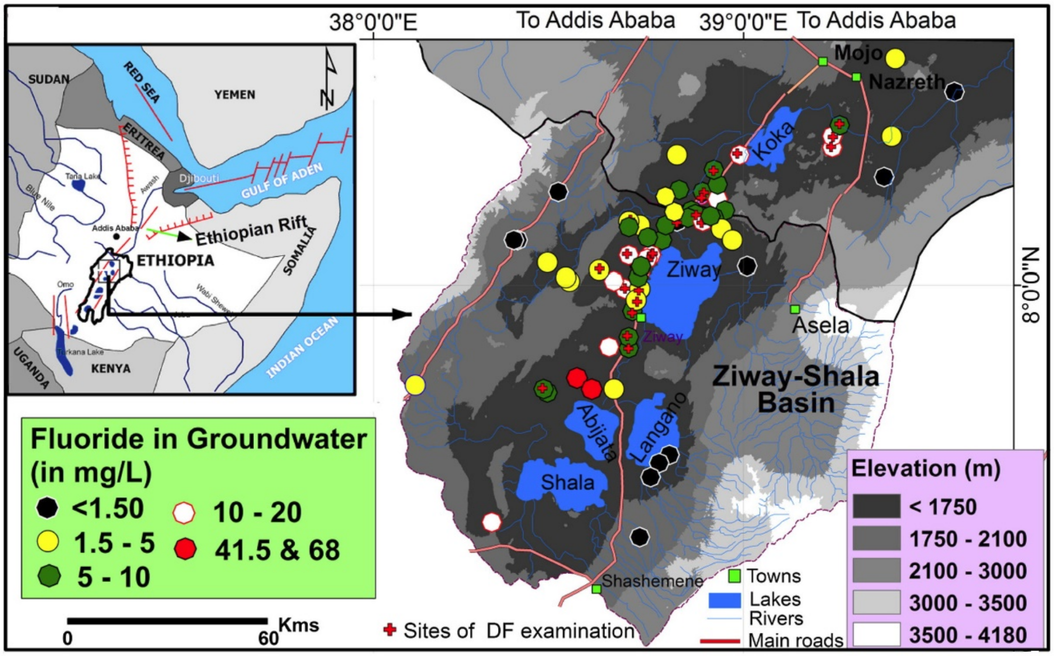

Figure 1. Groundwater sampling sites with measured F^- concentrations and sites that received DF examination. Modified from Rango et al. (2012).

2.1.2. Study population and dental fluorosis examination

Clinical examination of DF was conducted in children drinking water from 33 of the 94 sampled wells (selected to represent a wide range of F^- concentrations ranging between 1 and 18 mg/L) (Fig. 1). Upon arrival in a village, the study team spread the word with key village informants that children were being recruited for a health study related to water quality. Children were then enrolled if they volunteered (with parental consent) to participate in the study by coming to local clinics, schools, or other village-level meeting sites. Only children between 10 and 15 years old ($n = 491$) were included in the sample in order to examine DF in permanent teeth. Furthermore only lifelong residents drinking from community wells that were constructed before their birth were enrolled. Given the lack of viable alternative sources, these children's exposure to F^- should thus have been relatively stable and consistent throughout the critical period of enamel development as well as formation of permanent teeth.

The dental health impact of F^- was evaluated using the TF Index (Thylstrup and Fejerskov, 1978), an epidemiological index that best correlates the clinical appearance of various degrees of fluorosis to pathologic change in the enamel and has a range for severe forms of DF that is more sensitive than alternatives such as Dean's index (Fejerskov et al., 1996). TF scores are grouped into the following categories: healthy translucent teeth (score of 0), mild DF (scores of 1 and 2), moderate DF (scores of 3 and 4), and severe DF (scores between 5 and 9). Scores of 1–4 correspond to increasing white opaque areas on the enamel. The severe form begins with focal pitting, followed by increasing pitting on the white opaque enamel that progresses to confluent pitting, and then total loss of enamel and tooth deformation (Fig. 2). Prior to DF examination, the vestibular (buccal) surfaces of teeth were cleaned and dried with sterile gauze, and the teeth were examined under natural light.

Figure 2. Severe enamel fluorosis in a 14-year-old boy born and raised with 13 mg/L of F^- in groundwater in the MER. TF scores of the upper and the lower jaw from left to right are (7, 8, 9, 8, 8, 6) and (5, 7, 7, 5, 5, 7, 6, 5), respectively.

A total of 12,526 upper and lower jaw teeth were examined in the 491 examined individuals. Teeth with cavities or any sign of dental caries were excluded from the examination. The reliability of the TF scores for incisor teeth was reassessed by a separate examiner using photos of the teeth; this comparison yielded scores showing an acceptable level of agreement with those of the field examiner with no significant difference ($R^2 = 0.8$; $p > 0.05$).

All examined teeth were categorized in accordance with their mineralization and age of eruption, as either early-erupting (i.e., incisors and first molars) or late-erupting (i.e., canines, premolars, and second molars). They were also grouped into posterior (i.e., premolars, first molars, and second molars) and anterior teeth (i.e., incisors and canines). These groupings were used for the analyses of DF severity with respect to the F^- in the groundwater and urine.

2.1.3. Urine sample collection and analyses

For F^- exposure assessment, we tested urinary F^- concentrations from first morning void urine samples of 156 children (a subset randomly selected from the larger group of 491 children) selected from 17 community wells representing a wide range of F^- concentrations (1 to 18 mg/L). Urine samples were collected in acid-washed 60 mL ultra-cleaned polyethylene bottles. A semi-quantitative urinalysis test using Siemens Multistix 8SGH (results were confirmed with a pH meter) was carried out in-situ to measure urinary pH. The F^- concentration in urine was also measured using ISE following the procedure used for the water samples described above. Quality control was conducted using freeze-dried urine reference material (SERO210705; LGC Standards) concurrently with urine samples of the individuals. The accuracy of ISE F^- measurements for both urine and water standards ranged from98% to 102.5% relative to the standard. No F^-concentrations were below the detection limit of the F^- electrode (0.02 mg/L).

2.1.4. F^- in milk

In addition to the aforementioned analyses, fresh milk samples from cows were collected from villages with well water containing low to high F^- and were tested for F^- concentration. In these communities, cows often drink water from both rivers and wells. The F^- concentration in cow's milk was measured in-situ using ISE following a procedure similar to that used for the water samples.

2.2. Surveys

A pre-tested and translated survey questionnaire was conducted in face-to-face interviews with sample children and their parents. It included questions about sex, age, place of birth, exposure duration, drinking water sources, water intake per day, groundwater well drilling year, toothpaste use, infant formula consumption, breast feeding history, and basic health survey data on nutritional status and perceptions of health risks.

For the nutritional assessments, we took several anthropometric measurements and asked mothers' to report on the breastfeeding and infant formula intake of the sample children. Specifically, the anthropometric measurements included height and weight to calculate body mass index (BMI; weight (kg)/height (m^2)), mid-upper arm circumference (MUAC), and subscapular skinfold thickness (SST) measured for the nondominant arm.

Finally, the average water intake in a day was estimated with reference to a standard container used by the households in this region.

2.3. Statistical analysis

The database construction and basic statistical analyses were conducted using Microsoft Excel 2010 and the IBM SPSS statistical package version 22. Descriptive analyses were carried out using quartiles, means and standard deviations for continuous variables. Bivariate analyses were performed using t-tests. StataSE version 11 was used for multivariate regression analyses of the effects of F^- (in water and urine) on TF scores, controlling for potential modifiers (such as age, sex, BMI, and breast feeding duration). The statistical significance was set at $p < 0.05$.

2.4. Ethical considerations

The research design was conducted with the ethical approval (Protocol No. A0045 and A0741) of the Institutional Review Board (IRB) at Duke University. Permission to carry out the survey was also obtained from Addis Ababa University in Ethiopia and from local water bureaus in the study region. All children (and their parents) who willingly participated in the survey were provided with a written informed consent prior to enrollment and participation in the study. The anonymity of investigated subjects has been maintained.

3. Results

3.1. General characteristics

The average age of children was 12 years; approximately half (52.3%; $n = 257$) of the study participants were female (Table 1). Based on the WHO (2000) classification of BMI, most children were categorized as underweight with the 75th percentile falling below 17.4 kg/m^2 and a mean BMI of 16.4 ± 2.15 kg/m^2. The mean water consumption per day in children was 1.2 ± 0.4 L. The F^- concentrations in the groundwater and urine samples ranged between 1.1 and 18 mg/L and 1.1 and 39.8 mg/L, respectively. The interquartile ranges of the estimated daily F^- intake per day, F^- intake per body weight per day, and urinary F^- concentration were 6.7–12.2 mg/day, 0.19–0.37 mg/kg bw/day, and 6.7–15.6 mg/L, respectively. Nearly all children (97%; $n = 476$) thus ingested an estimated daily amount of F^- that exceeded the U.S. EPA's NOAEL value for F^- (0.06 mg/kg bw/day).

Table 1. Statistical descriptions of the characteristics and F^- exposures of children in the survey

			Percentiles				
	N	Min	25th	50th	75th	Max	Mean ± SD
Anthropometric measures							
Age	491	10.0	11.0	12.0	13.0	15.0	12.1 ± 1.6
Weight (kg)	487	19.0	28.0	32.0	39.0	61.0	34.1 ± 8.9
Height (m)	487	1.15	1.34	1.42	1.52	1.83	1.42 ± 0.1
BMI (kg/m^2)	486	11.2	15.0	16.2	17.4	25.8	16.4 ± 2.2
MUAC (cm)	484	10.0	17.0	18.5	21.0	28.0	19.0 ± 2.6
SST (mm)	354	7.0	10.0	10.0	12.0	22.0	10.5 ± 1.8
Water F^- concentrations							
Water intake (liter/day)	491	0.33	1.0	1.0	1.33	2.70	1.15 ± 0.4
F^- in groundwater (mg/L)	491	1.10	5.4	8.14	11.1	18.0	8.7 ± 3.9
F^- intake (mg/day)	491	0.54	6.7	8.77	12.2	36.0	9.8 ± 5.4
Dose (mg/kg bw/day)	491	0.01	0.19	0.27	0.37	1.03	0.23 ± 0.2
Milk F^- concentrations							
F^- in cow's milk	15	0.042	0.059	0.087	0.11	0.13	0.09 ± 0.03
Urinary measures							
F^- in urine (mg/L)	156	1.10	6.74	11.5	15.6	39.8	12.1 ± 7.3
Urinary pH	156	4.85	5.19	6.0	6.48	8.50	5.9 ± 0.85
Breast feeding history							
Breast milk alone (months)	355	1.0	6.0	6.0	6.0	12.0	6.3 ± 2.2
Start drinking water (months)	356	1.0	6.0	6.0	7.0	12.0	6.8 ± 2.3
Stop breast feeding (months)	355	3.0	24.0	24.0	24.0	60.0	24.8 ± 8.6

The measured urinary pH range was 4.9–8.5, with a mean of 5.9 ± 0.85. The F^- concentration in the cow's milk samples ranged between 0.04 and 0.13 mg/L, with a mean of 0.09 ± 0.03 mg/L. Among the sample children, the average length of exclusive breastfeeding duration from birth was between 6 and 7 months; by 25 months on average, children had ceased breastfeeding. Only 5% (n = 18) of the interviewed children consumed infant formula during childhood, and only two reported using toothpaste.

3.2. Fluoride in groundwater and dental fluorosis

Evidence of DF (TF scores ≥ 1) was observed in at least one tooth in all 491 children, indicating 100% DF prevalence for individuals drinking from the 33 groundwater wells containing F^- levels of 1.1 to 18 mg/L (Table 2). Severe dental health impacts were found in 45% of the examined teeth, exhibited varying degrees of loss of the enamel (TF scores of 5 to 9). The positive associations between F^- in drinking water and the prevalence of DF or mean severity of TF scores appear linear at first (Fig. 3A and B) but then level off with similar prevalence or severity (mostly TF scores of 5 and 6) in the teeth of individuals who consume drinking water with F^- concentration above ~6 mg/L (72% of the children examined in this study). Among all examined teeth within the subgroup of children consuming groundwater with concentrations above 6 mg/L, 43.7% were assigned TF scores of 5 and 6.

Table 2. Prevalence of DF (TF scores 0–9) by water F^- concentration in 10- to 15-year-old children in the MER

Community name	F^- in ground-water (mg/L)	Percent of teeth with each TF score										Mean TF scores of all teeth	Number of children	Number of teeth
		0	1	2	3	4	5	6	7	8	9			
Oda	1.06	40.0	41.3	15.0	3.8	0	0	0	0	0	0	0.8 ± 0.1	3	80
Sera	1.61	43.5	30.6	14.0	8.1	1.6	2.3	0	0	0	0	1.0 ± 0.9	17	444
Wedesha	2.92	32.3	21.5	24.7	13.3	3.7	3.5	1.0	0	0	0	1.5 ± 1.0	16	405
Beyimo	3.70	49.3	24.9	12.3	9.9	2.7	0.8	0	0	0	0	0.9 ± 0.8	15	373
Hezbawe	3.73	6.10	15	19.6	16.8	14.3	15.7	5.4	6.8	0.4	0	3.2 ± 1.4	11	279
Gebeba Rasa	4.00	17.2	19.7	19.3	16.8	16.0	5.7	2.5	2.5	0.4	0	2.4 ± 1.4	10	244
Edokontolla	5.20	12.8	4.8	7.4	8.2	23.1	20.5	14.4	8.0	0.8	0	3.9 ± 1.2	17	376
Tuchidako	5.24	1.6	11.7	10.5	16.6	28.4	19.1	10.3	1.9	0	0	3.6 ± 1.0	18	450
Hafa Rosa	5.27	8.8	7.7	17.9	25.0	21.7	14.8	2.5	1.6	0	0	3.0 ± 1.2	14	364
Haleku	5.40	0	2.1	2.4	10.7	16.8	30.3	15.6	12.5	6.1	3.4	5.2 ± 1.3	12	327
Wergaweshengula	5.42	10.8	7.2	16.8	30.5	19.2	7.2	7.2	1.2	0	0	2.9 ± 1.3	7	167
Elecametramofa	7.20	8.5	4.6	16.5	18.1	16.1	26.0	5.4	3.6	1.0	0.2	3.5 ± 1.3	8	497
Jido	7.20	1.8	0.9	10.0	16.8	15.0	22.3	13.2	15.5	3.6	0.9	4.7 ± 1.5	19	220
Orgacho	7.23	16.8	5.2	12.7	24.8	21.8	11.4	3.4	3.9	0	0	2.9 ± 1.2	17	440
Choreke	7.24	2.5	1.4	3.3	5.7	37.0	26.8	10.9	10.5	1.8	0	4.6 ± 1.2	18	488
Tejitu	7.84	0	4.3	3.9	11.7	19.9	46.3	13.0	0.9	0	0	4.4 ± 0.7	9	231
Berta	7.96	0	0.2	1.5	6.1	14.2	36.6	21.1	17.2	2.3	0.8	5.4 ± 0.7	19	507
Negaligne	8.14	4.8	3.4	11.2	10.8	20.5	19.3	12.2	13.3	4.6	0	4.4 ± 1.5	20	502
Bofo	8.60	0.6	0.9	4.6	8.9	18.3	35.6	22.0	8.9	0.4	0	4.9 ± 1.0	20	542
Tuchigabriel	8.73	0	0	2.9	5.8	19.6	51.4	18.8	1.4	0	0	4.5 ± 0.3	4	111
Aneno	8.77	0	3.6	9.1	19.4	19.4	30.9	15.2	2.4	0	0	4.1 ± 1.1	6	167
Wonji (Camp-3)	9.66	0	0.6	5.0	11.8	8.7	45.3	16.1	12.4	0	0	4.7 ± 0.8	7	188
Chore	9.88	0.8	2.8	7.9	9.3	23.1	19.8	17.0	16.8	2.4	0	4.7 ± 1.1	20	494
Sarete	10.4	4.4	4.4	6.2	5.6	23.9	24.5	15.3	11.5	3.8	0.3	4.6 ± 1.1	14	339
Tuchigrabona	10.7	0	0	1.3	13.9	26.0	33.6	11.3	13.4	0.4	0	4.8 ± 0.7	9	238
Wulumbula	10.8	4.7	4	10.2	14.9	19.6	21.5	13.2	9.2	1.5	1.2	4.2 ± 1.2	27	683

Gura	11.1	10.3	2.9	6.2	9.9	19.1	19.7	16.2	12.7	2.9	0.2	4.2 ± 1.3	21	487
Wodera	11.2	4.8	12.2	6.3	4.4	19.6	22.5	17.0	11.1	2.2	0.0	4.2 ± 1.5	12	289
Woyogabriel	11.3	0	0	7.6	4.1	14.9	50.8	19.5	1.6	1.6	0	4.8 ± 0.6	15	371
Wonji (camp-7)	13.0	1.9	3.1	4.5	7.3	13.2	23.7	11.3	20.6	11.5	2.8	4.4 ± 0.8	6	165
Wegea	13.2	8.4	6.3	11.2	13.4	24.4	24.2	9.2	2.4	0.2	0.1	3.6 ± 1.4	34	890
Wonji (camp-9)	13.3	0.2	2.2	4.0	8.9	13.3	32.2	10.9	23.5	4.0	0.8	5.3 ± 1.2	22	527
Cheleleki	18.0	1.3	1.3	2.2	5.2	12.8	30.6	14.7	21.7	8.1	2.2	5.5 ± 1.2	24	641

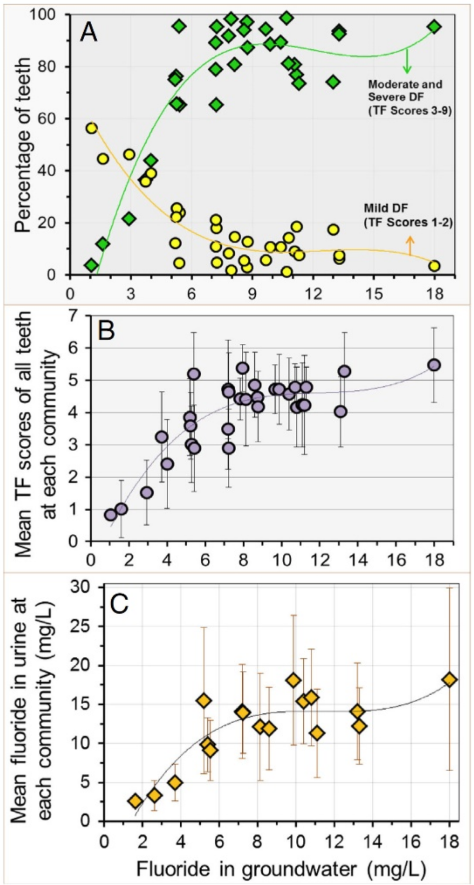

Figure 3. (A) Percentage of teeth with DF (mild and moderate-severe); (B) severity of DF; and (C) children's urinary F^- concentration; as a function of F^- concentrations in groundwater (See also Table 4). Each point is a community-level average. Percentage and severity of teeth with mild-moderate-severe (TF scores ≥ 1) and F^- concentrations in groundwater best fit by a 3rd order polynomial model. (A) Percentage of teeth with DF= $0.035\ F^3 - 1.3\ F^2 + 15.7\ F + 38.4$; $R^2 = 0.85$, and (B) DF severity= $0.0031\ F^3 - 0.11\ F^2 + 1.34\ F - 0.82$; $R^2= 0.78$, and (C) the F^- relationship in groundwater and urine is best fit by a 3rd order polynomial model [F^- in urine = $0.014\ F^3 - 0.49\ F^2 + 5.6\ F - 7.1$; $R^2 = 0.77$]; where F is F^- concentration in groundwater. Vertical lines indicate standard deviations from the mean within each community.

Most of the children exposed to groundwater F^- concentrations below 1.6 mg/L had healthy teeth (TF scores of 0) in some of their dentition; such scores were assigned to about 9% of all the teeth examined in the study. The mild, moderate, and severe DF prevalence in all examined teeth was 17%, 29%, and 45%, respectively. Furthermore, ~11% of the teeth exhibited TF scores of 7, 8, and 9. Thus, an assessment of the prevalence of aesthetically significant fluorosis (TF scores of ≥3) in the teeth of all children was 74%. Referencing the relationship shown in Fig. 3A, at the F^- level equivalent to the WHO drinking water standard of 1.5 mg/L, 53% and 5% of the teeth exhibited a mild and moderate form of DF, respectively. At the F^- level of 2 mg/L, equivalent to the U.S. EPA's SMCL standard, 47% of teeth showed mild DF, with the remaining teeth displaying moderate (14.7%) to severe (2.8%; predominantly with TF score of 5) enamel damage. At F^- level of 4 mg/L, equivalent to the U.S. EPA's MCLG standard, 28.5% of teeth showed mild DF, with the remaining teeth displaying moderate (28%) to severe (26%; predominantly with TF scores of 5 to 7) enamel damage. Thus, while the WHO standard predominantly corresponds to the mild form of dental impact in this population, neither of the U.S. EPA standards appears to guarantee a safe F^- threshold in the study population. The prevalence of moderate and severe DF approaches zero only at F^- concentrations below 1.2 mg/L and 1.8 mg/L, respectively.

The severity of DF also varies depending on tooth type and group (Table 3). Molars and incisors were more severely affected as compared to canines and premolars ($p < 0.001$). Severity of DF was significantly higher in the upper jaw teeth relative to the lower ones, with average TF scores of 4.1 ± 1.7 and 3.8 ± 1.7, respectively ($p < 0.01$). TF scores in early-erupting and posterior teeth were also greater in the upper jaw than in the lower jaw($p < 0.01$).

Table 3. Quartile measures of mean TF scores of individuals by tooth types and groups of teeth[a]

		Percentiles				
	N	25th	50th	75th	Max	Mean ± SD
Type of tooth						
All central incisors	491	3.0	4.0	5.0	8.0	4.0 ± 1.6
All incisors	491	3.0	4.0	5.0	8.0	3.9 ± 1.6
All canines	490	2.0	4.0	4.7	7.5	3.5 ± 1.9
All premolars	491	2.1	4.1	5.1	8.6	3.7 ± 2.0
All molars	481	3.8	5.3	6.0	8.8	4.8 ± 1.9
Group of teeth						
Upper jaw early erupting	491	3.3	4.7	5.5	8.2	4.4 ± 1.7
Upper jaw late erupting	491	2.2	4.1	5.1	8.4	3.8 ± 1.9
Posterior upper jaw	491	2.8	4.6	5.4	8.8	4.1 ± 1.9
Posterior lower jaw	491	2.5	4.4	5.5	9.0	4.0 ± 2.0
Anterior upper jaw	491	2.8	4.2	5.0	8.3	4.0 ± 1.7
Anterior lower jaw	491	2.3	3.8	4.7	7.3	3.5 ± 1.6
All early erupting	491	3.2	4.4	5.3	7.7	4.1 ± 1.6
All late erupting	491	2.3	4.1	5.2	8.2	3.8 ± 1.9
All upper jaw	491	2.9	4.4	5.2	8.0	4.1 ± 1.7
All lower jaw	491	2.8	4.1	5.0	7.7	3.8 ± 1.7
All teeth	491	2.9	4.3	5.1	7.8	3.9 ± 1.7

a. Note that minimum TF scores were zero for all tooth types and teeth groups. Fewer observation in canines and molars in some individuals is because of dental caries or not erupted teeth.

Table 4. Distribution of F^- in groundwater, urinary F^- concentration, pH in urine, and TF scores of all teeth

F^- in groundwater	F^- in urine[a]	pH in urine[a]	TF scores of all teeth[a]	Number of children
1.6	2.6 ± 0.8 (1.4–4.2)	6.1 ± 1.1 (5.0–8.5)	1.2 ± 1.0 (0–3.3)	9
2.6	3.3 ± 1.9 (1.2–6.9)	5.9 ± 1.4 (5.0–8.5)	1.0 ± 0.8 (0.12–2.7)	8
3.7	5.0 ± 2.4 (1.1–7.6)	5.0 ± 0.0 (5.0–5.0)	0.6 ± 0.2 (0.3–0.8)	8
5.2	15.5 ± 9.4 (4.4–29.2)	6.4 ± 0.6 (6–7.5)	4.4 ± 0.8 (3.1–5.4)	6
5.4	9.8 ± 3.5 (4.9–17.2)	5.8 ± 0.5 (5.2–6.6)	5.2 ± 1.3 (3.7–7.8)	12
5.5	9.1 ± 3.9 (3.1–16.2)	5.4 ± 0.6 (5.0–6.5)	3.2 ± 0.9 (2.4–4.8)	7
7.2	14.1 ± 6.0 (6.4–25.6)	6.5 ± 1.0 (4.9–7.5)	3.5 ± 1.3 (1.5–5)	10
7.2	13.9 ± 5.2 (8.4–24.9)	5.9 ± 0.4 (5.2–6.6)	4.2 ± 1.3 (1.1–6.4)	10
8.1	12.1 ± 6.9 (3.7–28.1)	6.4 ± 0.7 (6.0–8.0)	3.8 ± 1.3 (1.9–6.6)	9
8.6	11.9 ± 5.3 (2.1–24.1)	5.9 ± 0.4 (4.9–6.5)	5.1 ± 0.7 (4.1–5.9)	12
9.9	18.1 ± 8.3 (9.7–34.8)	6.4 ± 0.9 (5.0–8.5)	5.2 ± 0.9 (3.3–6.2)	10
10.4	15.4 ± 5.5 (5.6–25.5)	5.6 ± 0.8 (4.9–6.9)	4.7 ± 1.2 (2.3–6.7)	10
10.8	15.9 ± 6.2 (10.1–28.0)	6.4 ± 0.8 (6.0–8.0)	4.6 ± 1.5 (1.8–6.2)	9
11.1	11.3 ± 5.7 (5.2–18.9)	6.4 ± 1.2 (5.0–8.0)	4.3 ± 1.5 (1.9–6.5)	6
13.2	14.1 ± 6.2 (8.6–22.2)	5.8 ± 0.9 (5.0–7.5)	2.6 ± 1.9 (0.7–5.5)	7
13.3	12.2 ± 4.9 (4.2–20.8)	5.5 ± 0.3 (5.1–6.2)	5.3 ± 1.4 (2.2–7.1)	14
18.0	18.2 ± 11.7 (6.6–39.8)	5.9 ± 0.9 (5.0–7.5)	5.6 ± 1.6 (2.9–7.5)	9

a. Values are expressed as mean ± SD (minimum–maximum).

Means of DF severity in all tooth types and group of teeth were not significantly different between male and female children ($p < 0.05$). Age was positively related to overall TF scores ($p < 0.01$). BMI, SST, and MUAC did not show a significant correlation with TF scores. Duration of breastfeeding and age of onset of groundwater consumption were not associated with DF severity. The lack of sensitivity of DF severity to these factors is likely due to the lack of variation in these parameters among children included in the survey. The results of these mean comparisons were also generally consistent with findings from multivariate regression analysis, which did not indicate consistent patterns of significance for these potential modifiers of DF outcomes (see Supplemental material, Table S1).

3.3. Fluoride in groundwater, and urine, and dental fluorosis

The mean urinary F^- concentration of all 156 children was 12.1 ± 7.3mg/L (range: 1.1–39.8 mg/L), whereas the average F^- concentration in groundwater was 8.5 ± 4.1 mg/L (range: 1.1–18 mg/L). Notably, at lower F^- exposures (< ~6 mg/L), the F^- exposure and mean urinary F^- concentration at each community seem to fit a linear relationship, but the concentration tends to level off at higher groundwater F^- concentrations (Fig. 3C). A similar relationship is observed between F^- exposure and DF outcomes (Fig. 3B). Despite this relationship, there is considerable variation in the individual specific urinary F^- concentration and the respective groundwater F^- concentration to which they are exposed (Fig. 4).

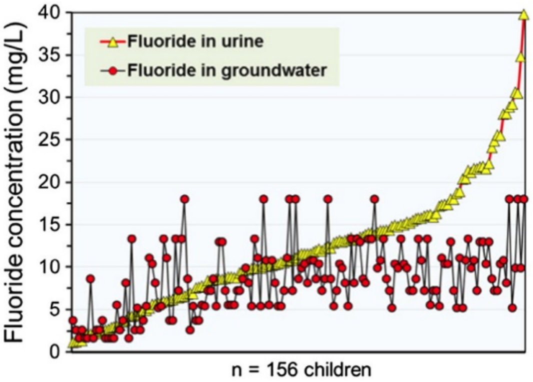

Figure 4. The association between F⁻ concentration in groundwater and the respective urinary F⁻ concentration for each child in the sample, ordered by urinary concentration.

3.4. Urinary fluoride concentrations after defluoridation

We tested urinary F⁻ concentrations in children from one community (Bofo site) where a pilot groundwater defluoridation project was being applied as part of remediation of the DF epidemic in the MER. As a result of the treatment, the measured F⁻ concentration in groundwater (originally 9 mg/L) was reduced by about 50% to 4.5 mg/L on average. Though we do not have pretreatment baseline measures of urinary F⁻ concentration, we measured lower urinary F⁻ concentration among tested subjects (*n* = 8) in the 10th month (7.3 ± 2.0 mg/L) compared to the 1st month (11.7 ± 2.2 mg/L) following the treatment (Fig. 5). This indicates that F⁻ assessment in urine could remain a useful tool for monitoring F⁻ exposure from other potential sources after defluoridation interventions. The persistence of relatively higher than expected F⁻ levels in urine suggests the possibility of other sources of F⁻ intake (e.g., locally grown food) or mobilization of F⁻ from skeletal tissues following a reduction in the level of F⁻ intake in treated groundwater as demonstrated in other studies (e.g., Whitford, 1999).

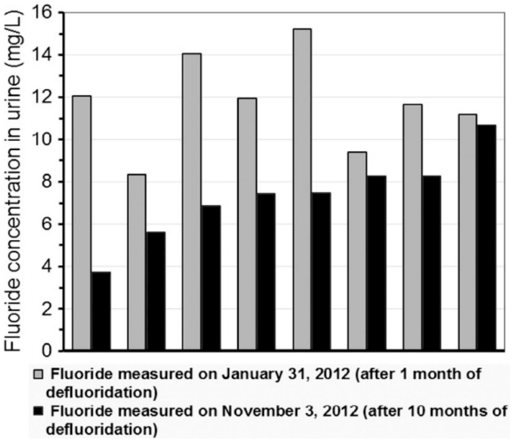

Figure 5. Change in children's (*n* = 8) urinary F⁻ concentration after defluoridation.

4. Discussion

This paper reports on findings from a cross-sectional study investigating the association between F^- exposure in groundwater, prevalence (and severity) of DF, and concentration of F^- excreted in urine. We limit risk of confounding by restricting the sample to a group of children (aged between 10 and 15) who are lifelong residents of communities with limited access to alternative water supplies. Among children drinking water from wells with concentrations above 6 mg/L, we find very high prevalence and severity of DF. We also find that groundwater F^- on the one hand, and DF and urinary concentrations of F^- on the other, are linearly and positively correlated up to this threshold. Beyond this threshold, both markers of high F^- exposure appear to level off. The study adds to previous research that has considered the relationship between groundwater F^- and DF. Finally, we provide some of the first evidence on F^- concentrations measured in urine for this region. As far as we know, no other studies in F^--endemic areas have linked such a wide range of F^- exposures to detailed analysis of DF in children.

Our study clarifies the relationship between F^- exposure and DF. Up to the F^- threshold of ~6 mg/L in drinking water, a linear relationship ($R^2 = 0.4$; $p < 0.001$) between F^- and DF appears valid. When F^- exceeds ~6 mg/L, however, the DF prevalence (and severity) levels off. The leveling off is most likely due to the fact that at ~6 mg/L the enamel is damaged as much as it can be clinically observed in most of the children. This indicates that the DF outcomes gradually become less pronounced with increased F^- exposures above this apparent threshold. Across the range of concentrations covered in this study, we found a very high prevalence of severe DF (45% of examined teeth), characterized by pitting and structural damage to teeth (TF scores ≥ 5). The mean TF scores at the lowest (1.1 mg/L) and highest (18mg/L) F^- concentrations in these communities were 0.8 ± 0.1 and 5.5 ± 1.2, respectively; the later DF severity is consistent with another study conducted in high F^- areas (9–14 mg/L) in the MER (Wondwossen et al., 2006).

We also found a similarly positive but nonlinear association (leveling off above ~6 mg/L of F^- in drinking water) between F^- concentrations in groundwater and mean urinary F^- concentration of children in each community. In addition, the majority (97%; $n = 152$) of the children tested for urinary F^- had higher daily F^- intake (range: 1.1–30 mg/day) from drinking water than the recommended daily dietary intake of F^- (2.5 mg/day) for children aged ≥ 10 years (U.S. NRC, 1989). It is interesting to note that a similar study of Tibetan children (8 to 15 years) found urinary F^- concentration of 1.8 mg/L corresponding to an average 5.4 mg/day F^- intake that resulted in a mild form of DF (Cao et al., 1996). In our study, most children's teeth showed mild DF at urinary F^- concentrations below 3 mg/L. We also found that a large proportion (90%; $n = 140$) of children had urinary F^- above 3mg/L, with some levels reaching up to 39.8 mg/L. These higher concentrations were predominantly associated with moderate to severe DF.

Children retain more F^- during growth and development of calcified tissues than later in life when net bone formation slows (Whitford, 1996, 1999). Considering the excessive and prolonged steady F^- exposure in the study population, we cannot rule out the possibility that calcified tissues for many individuals drinking high F^- groundwater are close to saturation with respect to this element, such that F^- uptake by calcified tissue is reduced and leads to a more significant proportion of F^- being excreted mainly through the kidneys. In this condition, the F^- excretion could exceed 80% of the total F^- intake (Myers, 1978).

Despite the similarity in the relationship between groundwater F^- concentration and community-level averages for DF or urinary F^- concentration, the latter outcomes were highly variable among the children within a community. Several factors may influence this variation in DF outcomes and urinary concentrations among the children, including genetic variation in susceptibility to enamel fluorosis, differences in total exposure from diets, variation in diets (e.g., food rich in protein and/or micronutrients), amounts of water consumed, urinary flow rate and urinary pH (Whitford, 1990; Yoder et al., 1998; U.S. NRC, 2006). Similar to Whitford (1990), we found that the urinary F^- concentration in MER children was positively correlated with urinary pH, though in our study this correlation was not significant ($p > 0.05$). Evidence from animal studies meanwhile suggests that individual genetic variability might contribute to susceptibility to enamel fluorosis (Everett et al., 2002; Carvalho et al., 2009).

The urinary F^- concentrations recorded in this research should account for F^- intake from dietary sources. We observed that the local communities in the MER heavily depend on subsistence agriculture and foods produced from locally grown crops (Malde et al., 2011; Rango et al., 2012). Very few children have access to infant formula, and thus F^- intake from infant formula is likely to be small or negligible in the majority of these children. In addition, almost none of the surveyed children use toothpaste, suggesting that the children were not exposed to F^- through incidental ingestion of toothpaste. Previous research has estimated that young children (< 5 years of age) living in the study area and consuming water with F^- concentrations of 2 mg/L and 14 mg/L ingest about 2.3 mg/day and 4.2 mg/day, respectively, from foods prepared using these water sources (Malde et al., 2004). In this study area, the total daily F^- intake in children consuming water with 2 mg/L is mainly derived from food (63%), while children consuming high-F^- water (14 mg/L) get most of their F^- through beverages (60%) (Malde et al., 2003). More recently, Malde et al. (2011) found that the daily F^- intake in children aged 2 to 5 years from water sources with 1.95 mg/L and 14.4 mg/L of F^-, was 34% and 50% of their total daily F^- intake of 3.1 ± 0.6 mg/day and 15.7 ± 2.9 mg/day (or 0.08 mg/kg bw/day, and 0.57 mg/kg bw/day), respectively (Malde et al., 2011). Similar calculations for F^- intake per kg of body weight are consistent with two villages in our study with similar F^- concentration of 1.6 mg/L and 13.4 mg/L, for which we estimated daily F^- intake of 2.2 ± 1.1, and 15.4 ± 7.5 mg/day (or 0.07 and 0.46 mg/kg bw/day). Another recent work from the study area has shown that the total F^- intake from drinking water alone among adults consuming groundwater with 1, 3, and 11.5 mg/L of F^- was 33%, 58%, and 86%, respectively (Dessalegne and Zewege, 2013). Our communities likely had similar dietary habits to both of these, so that the F^- contribution from food that is measured in these studies provides a potentially valid estimate of the amount of F^- individuals ingest from such sources.

Other dietary factors may also play a role. For example, earlier findings from the same region have suggested that children who consume cows' milk in the MER are somewhat less likely to have severe DF (on average about 10% lower TF scores; $p < 0.05$) (Rango et al., 2012; Kravchenko et al., 2014). As part of the current study, we measured the concentration of F^- in 15 samples of cows' milk and found it to be very low (mean: 0.09 ± 0.03 mg/L; range:

0.04–0.13 mg/L), consistent with results from another study conducted in Canada (mean F^- concentration: 0.041 mg/kg; range: 0.007–0.086 mg/kg) (Dabeka and McKenzie, 1995). A study in the Kenyan Rift Valley among nursing mothers consuming high F^- drinking water (9 mg/L) similarly found negligible levels of F^- in breast milk, ranging from 0.011 to 0.073 mg/L (Opinya et al., 1991). The low F^- level in milk, coupled with its high nutritional value (i.e., high calcium and magnesium), may provide protection against dental and skeletal fluorosis. In general, future studies in the MER should focus on further clarifying the total F^- intake from food and water sources, and consequently uptake by the skeleton, using methods such as 24-hour urinary excretion, and taking account of milk consumption.

The F^- levels in MER groundwater in our study sites are much higher than the WHO drinking water limit of 1.5 mg/L and are also higher than the U.S. EPA's primary standard of 4 mg/L. While the U.S. EPA has set this standard in order to minimize the risk of skeletal fluorosis, some studies have shown that mild skeletal effects may occur below 4 mg/L (Cauley et al., 1995; Ayoob and Gupta, 2006). Further investigation in these locations may provide important evidence on other adverse health conditions attributable to excessive F^- exposure, such as skeletal effects, which is not very well documented in the MER. For example, a study in Tanzania (part of the EARS) found SF in children (juvenile SF) exposed to high-F^- (up to 35 mg/L) concentrations in the groundwater (Jarvis et al., 2013). This finding suggests the potential for occurrence of similar juvenile SF cases in the MER region.

Furthermore, whereas surveys in the U.S. have found low prevalence of DF among children aged 12–15 years at the EPA standard (e.g., 37% very mild to mild DF, and 3.6% moderate to severe) (Beltran-Aguilar et al., 2010), we observed severe DF (enamel pitting) in 26% of children's teeth at this level. In milder forms of DF, it is only possible to observe changes in the enamel; however, the dentin can be affected in more severe cases (Fejerskov et al., 1996) such that protection against decay and infection is compromised. This can cause tooth sensitivity (TS) and affect eating, drinking, and breathing through the mouth (Mine et al., 2011; Zhang et al., 2014). Moderate and severe DF is also associated with increased caries and psychological and social impacts (Wondwossen et al., 2006). Thus, severe DF affects tooth function and overall quality of life of afflicted individuals, rather than simply having cosmetic effects.

4.1. Limitations of the study

We selected communities representing the range of groundwater F^- concentrations and recruited a volunteer sample rather than conducting a random population-based sampling. The study thus considers only a limited age range and a range of concentrations to which individuals in the region are exposed. Due to lack of time-series measurements, water quality sampling was conducted during the dry season to minimize seasonal variations arising from varying recharge across wells. Still, we do not expect significant variation in the water chemistry in the deep (> 50 m) aquifer system underlying the MER (Rango et al., 2013), from which the majority of study households obtain their drinking water. In addition, though groundwater was the sample population's primary water source, alternative water sources with low F^- (e.g., surface waters during rainy season, or piped water from neighboring towns) may be available and used intermittently by some households. In addition, although fluorosis occurs as a result of cumulative F^- exposure, our survey only measured DF prevalence at a single point in time; we therefore do not observe how the severity of DF in individuals evolves over time. Similarly, due to time and logistical constraints imposed by the dispersed nature of the study communities, we obtained only early morning spot urine samples, and thus reported F^- concentrations from these samples rather than excretion over a 24-hour urine, which provides a more reliable estimate of F^- exposure than spot urine (Zohouri et al., 2013). Finally, data on breastfeeding and consumption of infant formula were obtained from the children's mothers' retrospective self-reports, which may vary in accuracy.

5. Conclusion

A significant proportion of the children examined in this study drinks water from sources with high levels of F^-, excretes urine with high levels of F^-, and suffers from dental health damage in the form of severe DF. The results show nonlinear positive relationships between F^- exposure, urinary F^-, and DF in children. In drinking water samples collected during the study, we did not find a minimum F^- concentration threshold below which DF was absent. However, we estimated two NOAELs, corresponding to F^- concentration thresholds of 1.2 and 1.8 mg/L in drinking water, as the lowest concentrations associated with no occurrence of moderate and severe DF in children's dentition, respectively. The findings from this study should be useful for planning preventive public health interventions such as education and behavior changes, or selecting sites from highly affected areas of the MER for promotion of water defluoridation and substitution. In addition, measurement of F^- concentration in urine provides a useful tool for evaluating human exposure to F^- that could help in monitoring the success of such interventions. We recommend that governments and nongovernmental organizations use this information for risk assessment and design of F^- exposure mitigation to better safeguard the health of populations in the MER and in other areas with high prevalence of fluorosis.

Abbreviations: bw, body weight; BMI, body mass index; DF, dental fluorosis; EARS, East African Rift System; F^-, fluoride; IRB, Institutional Review Board; ISE, Ion Selective Electrode; mg/L, milligram per liter; mg/kg bw/day, milligram per kilogram body weight per day; MER, Main Ethiopian Rift; MCLG, Maximum-Contaminant-Level Goal; MUAC, mid-upper arm circumference; NOAEL, No-Observed-Adverse-Effects-Level; SMCL, Secondary-Maximum-Contaminant-Level Goal; SST, subscapular skinfold thickness; TISAB, Total Ionic Strength Adjuster Buffer; TF Index, Thylstrup and Fejerskov Index; U.S. NRC, U.S. National Research Institute; U.S. EPA, U.S. Environmental Protection Agency; WHO, World Health Organization.

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Authors' contributions: Tewodros Rango: Designed the study concept; conducted field work, analytical data analysis, and interpretation; and wrote the manuscript.

Avner Vengosh: Designed the study concept and critically revised the manuscript for important intellectual content.

Marc Jeuland: Interpreted data and critically revised the manuscript for important intellectual content.

Redda Tekle-Haimanot, Erika Weinthal, Julia Kravchenko, Christopher Paul, and Peter McCornick: Assisted with field work and revised the manuscript for important intellectual content.

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Supplemental Material

Supplemental Material, Table S1. Relationship between F⁻ in groundwater and average TF scores for all teeth and early-erupting teeth only, and urinary F⁻ concentration, controlling for potential mediating factors[a]

	All teeth	Early erupting teeth	Urinary F⁻ concentration
ln(F⁻)	1.72*	1.79*	6.38*
	0.24	0.25	0.74
Female	0.04	–0.03	0.94
	0.13	0.14	0.99
Age	0.24*	0.07	–0.40
	0.06	0.07	0.46
MUAC	0.02	–0.01	0.00
	0.04	0.05	0.24
constant	–2.85*	–0.13	3.40
	0.80	0.82	5.43
N	486	486	156
R-squared	0.40	0.36	0.30

a. Models including duration of breastfeeding or age of onset of drinking groundwater (and interactions with F⁻ levels expressed in log-term) did not lead to substantive changes in results or improvements in model fit. Standard errors were shown on second line. * = $p < 0.01$.