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Research Article

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Abstract

Background: Ingestion of fluoride in drinking water has been shown to result in increased cellular markers of inflammation in rodent models. However, the approximately 10x greater fluoride concentrations in drinking water required by rat and mouse models to obtain blood fluoride concentrations similar to those found in humans, has made the relevance of these animal studies difficult to assess. Increased white blood cell count is a marker of inflammation in humans, and therefore we used available 2013-2016 NHANES survey data, which includes both blood cell counts and plasma fluoride concentrations, to explore the relationship between fluoride and inflammation in humans.

OBJECTIVE: To assess associations between plasma fluoride levels and blood cell markers of inflammation in a US population.

METHODS: Multiple linear regressions to determine the association of blood cell counts and plasma fluoride were done using publicly available NHANES survey data from the 2013-2014 and 2015-2016 cycles. Plasma fluoride concentrations were available for children aged 6 to 19, and therefore this subpopulation was used for all analyses. Covariate predictors along with plasma fluoride were age, gender, and Body Mass Index (BMI).

RESULTS: Plasma fluoride was significantly positively associated with water fluoride, total white blood cell count, segmented neutrophils, monocytes, and negatively associated with red blood cell count when adjusted for age, gender and BMI.

CONCLUSION: Our finding that neutrophils and monocytes are associated with higher plasma fluoride in US children and adolescents is consistent with animal data showing fluoride related effects in increasing inflammation. These findings suggest the importance of further studies to assess potential mechanisms for these fluoride related effects in tissues and organs, such as the small intestine, liver and kidney, that are involved in absorption and filtration of ingested fluoride.

Background

Fluoride is a highly electronegative anion which, when present in saliva or other topical dental products, enhances the precipitation of calcium phosphates on the tooth enamel surface (ten Cate and Featherstone 1991). The observation that naturally fluoridated water was associated with reduced dental decay (Dean 1956), lead to recommendations to add fluoride to drinking water as a public health measure to prevent dental caries (Health and Human Services Federal Panel on Community Water 2015). Current estimates, posted by the US Centers for Disease Control and Prevention (CDC), are that 73% of community water systems in the US provide fluoridated water, and 63% of the US population receives fluoridated water (https://www.cdc.gov/fluoridation/statistics/2018stats.htm).

When ingested, fluoride is first partially absorbed (approximately 25%) through the stomach in the form of hydrofluoric acid (Whitford and Pashley 1984), and the remainder is absorbed in the small intestine, in a process independent of pH (Buzalaf and Whitford 2011). Following absorption from the stomach and small intestine, fluoride is further filtered by the liver and kidney. This results in plasma fluoride levels in humans that are approximately 10-fold less than the concentration in drinking water. However, higher fluoride concentrations (approximately 10x) are required in drinking water of mice and rats to achieve plasma fluoride levels similar to those of humans.

This concentration differences in fluoride in drinking water and plasma fluoride in rodents as compared to humans, is likely due to anatomical difference that affect fluoride clearance. The length of the mouse small intestine per body weight is much longer that human and in the mouse, with overall transit time approximately 10x faster than humans (Hugenholtz and de Vos 2018), resulting in a more rapid movement of fluoride out of the small intestine to the liver and kidney. In the kidney, the mouse has nearly 100 times fewer nephrons as compared to human nephrons (O’Brien, Guo et al. 2016, Jain 2017), resulting in lower glomerular filtration with a resultant lower plasma fluoride concentration. These differences in rodent models as compared to humans, have complicated the interpretation of fluoride effects using rodent model systems.

In rats, drinking water containing 10 or 25 ppm fluoride results in increased inflammation of the duodenum (Melo, Perles et al. 2017), jejunum (Dionizio, Melo et al. 2018), and illeum (Dionizio, Uyghurturk et al. 2021), with histological changes similar to
that of Crohn's disease. In Crohn's disease inflammation of the human small intestine is associated with increased white blood cell counts.

White blood cell counts are a reliable marker of inflammation (Wirth, Sevoyan et al. 2018), and therefore, to assess the relevance of fluoride-related increases in inflammation in rats and mice, to findings in humans, we utilized data from the National Health and Nutrition Examination Survey (NHANES). Publicly available data from NHANES surveys collected during the 2013–2014 and 2015–2016 cycles includes both plasma fluoride concentrations and complete blood cell counts (CBC) for children aged 6 to 19, and therefore we used these data to determine the association between plasma fluoride and blood cell counts in children and adolescents.

**Materials And Methods**

Plasma fluoride concentrations for both the 2013–2014 and 2015–2016 cycles of the NHANES survey were measured by the same laboratory at the College of Dental Medicine, Georgia Regents University, Augusta, GA. Fluoride was measured using an ion-specific electrode following hexamethyldisiloxane (HMDS) diffusion. To ensure the greatest accuracy in our analyses, only samples with reported measurable fluoride (at or above the detection limit) were included in our analyses, and assumed fluoride levels at the lower limit of detection limits (LLLOD) were excluded. To focus most specifically on fluoride levels relevant to community water fluoridation, we excluded the relatively small number of high outlier plasma fluoride levels greater than 5 micromolar fluoride.

Sampling weights and variance correction variables were used when analyzing the combined 2013–2014 and 2015–2016 NHANES survey data to account for the NHANES survey design as recommended by the National Center for Health Statistics (NCHS). The subpopulation was defined as those aged 6 to 19 years whose fluoride plasma comment code was 0 (at or above the detection limit), and whose plasma fluoride value was less than or equal to five µmolar.

Predictors that have been shown to increase white blood cell counts in children include age (Li, Peng et al. 2020) and body mass index (BMI) (Jeong, Lee et al. 2022). We therefore included age and BMI as covariates, along with gender, as women have been shown to have lower leukocyte counts than men (Chen, Zhang et al. 2016). We considered also including the family poverty to income ratio (PIR) as an indicator of socioeconomic status. However, this measure was not statistically significant in any of the models, and furthermore, its inclusion was not supported by published literature, so this variable was not included in any of our linear regression models. Dependent variables tested in this regression model were blood cell counts available in these same subpopulations.

**Statistical analyses**

All analyses applied survey weights from the mobile exam center visit (i.e. MEC weights) and the strata and PSU variables to account for the stratified clustered sampling design and to permit generalization to the U. S. population (National Center for Health Statistics, 2013). Descriptive statistics and regression analyses were performed using Stata 17.0 software. Survey-weighted linear regression was used to model blood cell counts as a function of plasma fluoride concentrations while adjusting for covariates (e.g., gender, age and BMI).

**Results**

The overall mean and standard deviation plasma fluoride level for this population was 0.46 +/-0.01 µmolar and ranged from 0.025 to 4.32 µmolar. Water fluoride levels ranged from 0.07 to 1.12 mg/L.

The results of analyses for total white blood cell count, individual white blood cell types, and other blood cell counts with significant associations with plasma fluoride concentrations are shown in Table 1. Shown in Table 1 are coefficients, 95% upper and lower confidence intervals (brackets) and the p values for each predictor (plasma fluoride, age, gender and BMI). Non-significant associations (p > 0.1) were found for hemoglobin, hematocrit, mean cell hemoglobin concentration, red cell distribution width, platelet count and mean platelet volume, and are not shown.
Plasma fluoride was significantly positively associated with water fluoride concentrations ($p < 0.001$), and white blood cell counts ($p = 0.018$). Among the different types of white blood cells, neutrophils (neutro) ($p = 0.033$) and monocytes (mono) ($p = 0.006$) were significantly positively associated with plasma fluoride concentrations, whereas lymphocytes (lymph), eosinophils (eosino) and basophils (baso) were not.

In regression analyses of other blood cell count values, we found red blood cell count (RBC) negatively associated with plasma fluoride concentrations ($p = 0.052$) and mean cell hemoglobin (MCH) positively associated with plasma fluoride concentration ($p = 0.060$).
<table>
<thead>
<tr>
<th>Table 1*</th>
<th>Plasma F</th>
<th>Age</th>
<th>Female</th>
<th>BMI</th>
<th>Constant</th>
<th>N</th>
<th>N subpop (raw)</th>
<th>N subpop (weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dep. variable</td>
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<td>WBC</td>
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<td>-0.089</td>
<td>0.395</td>
<td>0.094</td>
<td>5.791</td>
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<td>3,453</td>
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<td></td>
<td>(0.087, 0.881)</td>
<td>(-0.121, -0.055)</td>
<td>(0.211, 0.579)</td>
<td>(0.072, 0.116)</td>
<td>(5.370, 6.212)</td>
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<tr>
<td></td>
<td>p = 0.018</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
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<tr>
<td>Lymph</td>
<td>0.076</td>
<td>-0.072</td>
<td>0.139</td>
<td>0.016</td>
<td>2.947</td>
<td>38,659,063</td>
<td>3,447</td>
<td></td>
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<td></td>
<td>(-0.047, 0.198)</td>
<td>(-0.080, -0.064)</td>
<td>(0.074, 0.205)</td>
<td>(0.012, 0.021)</td>
<td>(2.785, 3.108)</td>
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<td></td>
<td>p = 0.217</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Neutro</td>
<td>0.366</td>
<td>-0.001</td>
<td>0.305</td>
<td>0.070</td>
<td>1.954</td>
<td>38,715,039</td>
<td>3,453</td>
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<tr>
<td></td>
<td>(0.031, 0.701)</td>
<td>(-0.029, 0.027)</td>
<td>(0.173, 0.437)</td>
<td>(0.052, 0.088)</td>
<td>(1.639, 2.270)</td>
<td></td>
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<tr>
<td></td>
<td>p = 0.033</td>
<td>p &lt; 0.938</td>
<td>p &lt; 0.000</td>
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<td>p &lt; 0.000</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mono</td>
<td>0.044</td>
<td>-0.004</td>
<td>-0.006</td>
<td>0.007</td>
<td>0.469</td>
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<td>3,447</td>
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<tr>
<td></td>
<td>(0.013, 0.075)</td>
<td>(-0.007, -0.002)</td>
<td>(-0.021, -0.008)</td>
<td>(0.005, 0.008)</td>
<td>(0.431, 0.506)</td>
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<tr>
<td></td>
<td>p = 0.006</td>
<td>p &lt; 0.001</td>
<td>p = 0.370</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
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<tr>
<td>Eosino</td>
<td>0.003</td>
<td>-0.010</td>
<td>-0.037</td>
<td>0.001</td>
<td>0.376</td>
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<td>3,447</td>
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<tr>
<td></td>
<td>(-0.025, 0.018)</td>
<td>(-0.014, -0.007)</td>
<td>(-0.054, 0.020)</td>
<td>(-0.001, -0.002)</td>
<td>(0.332, 0.420)</td>
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<tr>
<td></td>
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<td>p &lt; 0.001</td>
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<tr>
<td>Baso</td>
<td>0.003</td>
<td>-0.001</td>
<td>-0.002</td>
<td>0.001</td>
<td>0.0398</td>
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<td>3,447</td>
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<tr>
<td></td>
<td>(-0.007, 0.012)</td>
<td>(-0.002, 0.001)</td>
<td>(-0.006, 0.003)</td>
<td>(0.000, 0.001)</td>
<td>(0.031, 0.048)</td>
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<tr>
<td></td>
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<td>p = 0.514</td>
<td>p = 0.002</td>
<td>p &lt; 0.001</td>
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<tr>
<td>MCH</td>
<td>0.339</td>
<td>0.194</td>
<td>-0.090</td>
<td>-0.063</td>
<td>27.543</td>
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<td>3,453</td>
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</tr>
<tr>
<td></td>
<td>(-0.015, 0.693)</td>
<td>(0.173, 0.215)</td>
<td>(-0.224, 0.049)</td>
<td>(-0.075, 0.051)</td>
<td>(27.253, 27.833)</td>
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</tr>
<tr>
<td></td>
<td>p = 0.060</td>
<td>p &lt; 0.001</td>
<td>p = 0.199</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td></td>
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</tr>
<tr>
<td>RBC</td>
<td>-0.059</td>
<td>0.016</td>
<td>-0.293</td>
<td>0.007</td>
<td>4.546</td>
<td>38,715,039</td>
<td>3,453</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-0.119, 0.001)</td>
<td>(0.012, 0.021)</td>
<td>(-0.325, -0.260)</td>
<td>(0.005, 0.010)</td>
<td>(4.454, 4.638)</td>
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<tr>
<td></td>
<td>p = 0.052</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
<td>p &lt; 0.000</td>
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<tr>
<td>Water</td>
<td>0.284</td>
<td>-0.000</td>
<td>-0.003</td>
<td>0.003</td>
<td>0.413</td>
<td>37,854,276</td>
<td>3,396</td>
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<td></td>
<td>(0.156, 0.413)</td>
<td>(-0.011, 0.003)</td>
<td>(-0.03, 0.024)</td>
<td>(-0.002, 0.001)</td>
<td>(0.267, 0.558)</td>
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<tr>
<td></td>
<td>p &lt; 0.001</td>
<td>p = 0.269</td>
<td>p = 0.804</td>
<td>p = 0.253</td>
<td>p &lt; 0.001</td>
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</table>

Discussion
Our finding of a significant positive association between plasma fluoride concentrations of humans and fluoride in the water that they drink, is similar to our previous findings of serum and water fluoride levels in pregnant women in northern California (Abduweli Uyghurturk, Goin et al. 2020). Our finding of a significant positive association between white blood cell counts in children aged 6-19 and plasma fluoride concentrations suggests an association between fluoride exposure and increased inflammation. It is of particular interest that plasma fluoride is positively associated with increased numbers of neutrophils and monocytes, but not with lymphocytes, eosinophils and basophils. Neutrophils and monocytes are the white blood cells that are recruited to sites of tissue damage (Kratofil, Kubes et al. 2017, Wang 2018), and suggests the possibility that plasma fluoride may be associated with tissue specific inflammatory changes.

The small intestine is one such possible site. In rats, fluoride ingested in drinking water results in lesions similar to those found in Crohn's disease (Melo, Perles et al. 2017, Dionizio, Melo et al. 2018, Dionizio, Uyghurturk et al. 2021), and increased neutrophils (Follin-Arbelet and Moum 2016), and monocytes (Mowat and Bain 2011) are key players in the chronic inflammation of Crohn's disease patients. It was interesting that red blood cell count was negatively associated with plasma fluoride (p=0.052). Reduced red blood cell counts resulting in anemia are also found in Crohn's disease, associated with bleeding into the digestive tract and/or poor absorption of B12 and folic acid (Guagnozzi and Lucendo 2014), which are required for the synthesis of red blood cells (Koury and Ponka 2004). Consistent with the possibility of reduced red blood cell synthesis, is our finding of increased mean cell hemoglobin (p=0.06). Increased hemoglobin per red blood cell could reflect decreased red blood cell synthesis.

The stomach is another possible site affected by fluoride, as ingestion of higher concentrations of fluoride, such as those resulting from the application of a 3.5% topical fluoride gel for caries prevention, can cause mucosal injury to the gastric epithelium in humans (Spak, Sjostedt et al. 1990). In animal models the kidney (Luo, Cui et al. 2017), and liver (Chen, Kuang et al. 2019) are also susceptible to increased fluoride related inflammation, and fluoride is associated with changes in liver and kidney parameters in US adolescents (Malin, Lesseur et al. 2019).

Though we adjusted for gender in our regression analysis, previous analyses of NHANES data sets show that males aged 6 to 19 have relatively higher plasma fluoride levels relative to water fluoride concentrations (Jain 2017). This may be due to differences between males and females in fluoride absorption by the kidneys (Sabolić, Asif et al. 2007) and suggests the possibility that the effects of water fluoridation such as those related to inflammation be influenced by relatively higher plasma fluoride levels in males, along with other gender related differences.

We considered assessing other markers of inflammation. However, C-reactive protein, a known marker of inflammation was measured only in the 2013-2014 NHANES survey, and high C-reactive protein was measured only in the 2015-2016 NHANES survey, and so were not included in our analyses. Measures of inflammatory cytokines were also not available for analyses in this data set.

**Conclusions**

Our findings of an association between plasma fluoride concentrations and increased white blood cell counts, suggests that ingested fluoride may be an environmental risk factor, which can contribute to systemic inflammation. Dental fluorosis, a biomarker for fluoride exposure, has continued to increase in the US (Neurath, Limeback et al. 2019, Dong, Yang et al. 2021), and as chronic inflammation is a significant risk factor for a multitude of diseases (Houser and Tansey 2017), these effects of fluoride may be particularly relevant to US populations. While these fluoride related effects may not be a risk factor for healthy individuals drinking fluoridated water, the possible contributions of fluoride ingestion to those with risk factors for inflammation, is an important consideration for further study.

**Declarations**

Declarations of interest: none

Ethics approval and consent to participate
Consent for publication
Not applicable.

Availability of data and materials
The datasets used for these analyses are publically available (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
DAU and CW compiled data on community water fluoridation, completed data analyses and contributed to the writing of the manuscript. PKD coordinated the study, and contributed to the writing of the manuscript.

Acknowledgements
Not applicable.

References


