Fluoride exposure in community prevention programmes for oral health using nail clippings and spot urine samples: A systematic review and meta-analysis.

ESKANDARI, Fatemeh, KUMAH, Elizabeth Adjoa, AZEVEDO, Liane <http://orcid.org/0000-0001-9966-9414>, STEPHENSON, John, JOHN, Sherley and ZOHOORI, Fatemeh Vida

Published version

ESKANDARI, Fatemeh, KUMAH, Elizabeth Adjoa, AZEVEDO, Liane, STEPHENSON, John, JOHN, Sherley and ZOHOORI, Fatemeh Vida (2023). Fluoride exposure in community prevention programmes for oral health using nail clippings and spot urine samples: A systematic review and meta-analysis. Caries research, 57 (3), 197-210.

Fluoride exposure in community prevention programmes for oral health using nail clippings and spot urine samples: a systematic review and meta-analysis

Short title: A Systematic Review with Meta-analysis of Biomarkers of Fluoride Exposure

Fatemeh Eskandari[1], Elizabeth Adjoa Kumah[1,2], Liane Azevedo[3], John Stephenson[3], Sherley John[1], Fatemeh Vida Zohoori[1,*]

[1] School of Health and Life Sciences, Teesside University, United Kingdom

[2] Department of International Public Health, Liverpool School of Tropical Medicine (LSTM)

[3] University of Huddersfield

***Corresponding author**

School of Health and Life Sciences, Teesside University, United Kingdom

E-mail: v.zohoori@tees.ac.uk

Phone: + 44 (1642) 342973

Number of Tables: 4

Number of Figures: 4

Word count: 4813

Keywords: Fluoride, Dental caries, Spot urine, Nail clippings, Biomarker

Abstract

Due to practical difficulties in quantifying fluoride exposure in populations, practical and accurate biomarkers can play a major role in the surveillance of fluoride. Among different fluoride biomarkers, spot urine and nail-clippings have gained more attention due to their ease of acquisition. However, there is no robust consensus about the accuracy of these biomarkers for the estimation of fluoride exposure. This systematic review and meta-analysis aimed to synthesize evidence on the association between fluoride exposure and the fluoride concentration of spot urine and nail-clippings. This review was conducted and reported using the PRISMA Statement. Nine databases (Medline, CINAHL, Web of Science, Scopus, ScienceDirect, Sage Journals Online, Campbell Collaboration, Cochrane Collaboration, and Embase); search engines (Google and Google Scholar); and grey literature were searched up to September 2022. All screening, data extraction, and quality assessments were conducted in duplicate. All experimental and observational research studies that reported the correlation between fluoride exposure and fluoride concentrations of spot urine and/or nail clippings were included. The Mixed-Methods Appraisal tool was used to assess the methodological quality of the included studies. A random effect meta-analysis was carried out to determine the relationship between fluoride exposure and fluoride concentration of biomarkers (i.e., spot urine and nail clippings). Forty-four studies met the inclusion criteria. A total of 694,578 participants were included in this review. Twenty-five studies were included in the meta-analysis. The primary meta-analysis showed a moderate correlation of 0.674 (95%CI: 0.623-0.725, n=25) between fluoride intake and fluoride concentration of spot urine and a strong correlation of 0.938 (95%CI: 0.520-1.355, n=11) between fluoride intake and the fluoride concentration of nail-clippings in all age groups. The findings of secondary meta-analyses showed a strong positive correlation between fluoride intake and fluoride/creatinine ratio of spot urine in children (0.929; 95%CI: 0.502-0.991; n=2). In conclusion, spot urine and nail-clippings have the potential to be employed as non-invasively obtained biomarkers in populations. However, due to the scarcity of high-quality, relevant studies, more research is needed to establish the validity of these biomarkers.

Introduction

Fluoride, the ionic form of fluorine, is a natural component of the biosphere and is found in water, soil, and air in varying amounts [Zohoori and Duckworth, 2020]. Despite being present in trace amounts in the body, it has a public health importance due to its role in bone and teeth mineralisation. Fluoride has been well-recognised for the prevention and control of dental caries, which is still the most predominant preventable health condition worldwide [Zohoori and Duckworth, 2020]. Fluoridation schemes such as water-, milk- and salt-fluoridation have been endorsed by many countries to prevent dental caries. However, during critical periods of tooth development (i.e., the first 6 years of life) excessive exposure to systemic fluoride can result in the development of dental fluorosis [O'Mullane et al., 2016]. Hence, there is a clear recommendation for monitoring fluoride exposure, particularly in children, before and after introducing any fluoridation or supplementation programme for the prevention of dental caries [WHO, 2014].

The ingested fluoride is mainly absorbed in the stomach and small intestine. The absorbed fluoride is circulated in the body via plasma and incorporated mostly into the calcified tissues, containing 99% of body fluoride. Kidneys are the main route of removal of fluoride from the body, with almost half of the daily absorbed fluoride excreted in the urine [Zohoori and Duckworth, 2020].

Since diet (including water) and unintentional ingestion of fluoridated dentifrices are the main sources of fluoride intake in children, it is extremely difficult to quantify fluoride intake from these multiple sources. Therefore, biological markers of fluoride can be of value for identifying and monitoring deficient or excessive fluoride intake.

Considering the body burden of fluoride, the biomarkers of fluoride exposure have then been divided into three categories [Pessan and Buzalaf, 2011, Rugg-Gunn et al., 2011, Lavalle-Carrasco et al., 2021]: contemporary (e.g., blood/plasma, saliva, and urine), recent (e.g., nails and hairs) and historical (e.g., bone and teeth). Contemporary biomarkers measure present or very recent exposure to fluoride, whereas recent and historical biomarkers measure sub-chronic and chronic exposure to fluoride [Pessan and Buzalaf, 2011, Rugg-Gunn et al., 2011, Lavalle-Carrasco et al., 2021]. Based on pharmacokinetic findings [Villa et al., 2010], 24-hour urinary fluoride excretion is considered a reliable biomarker of fluoride exposure [WHO, 2014]. However, it is extremely difficult to collect 24-hour urine samples from children, especially in younger age groups who are not toilet-trained. As alternatives to 24-hour urine, spot urine and nail clippings are the most studied biomarkers of fluoride exposure due to being non-invasive, ease of acquisition, and their acceptability by study participants [Idowu et al., 2020, Idowu et al., 2021]. In order to establish the reliability of any biomarkers, it is imperative to better understand their associations with fluoride exposure. Our recent scoping review [Kumah et al., 2022] ascertained the nature and extent of the available evidence on how spot urine and nail clippings, as

alternatives to 24-hour urine, were used to measure fluoride intake/exposure by mapping the available literature according to their study population, setting, type of study design, methodology, and analytical approach. The review identified 55 articles in which associations between fluoride intake (and/or 24-hour urinary fluoride excretion) and a fluoride biomarker (spot urine and/or nail clippings) were reported, showing that there is enough evidence to explore the association between fluoride intake and fluoride biomarkers to be synthesised in a systematic review. This follow-up systematic review with a meta-analysis aimed to answer the following primary research questions: what is the relationship between fluoride exposure and fluoride concentration of: (i) spot urine; and (ii) fingernail/toenail fluoride?

The review also aimed to answer the following secondary research questions: what is the relationship between fluoride exposure and: (i) fluoride/creatinine ratio of spot urine; and (ii) fluoride/specific gravity ratio of spot urine?

Methods

This systematic review and meta-analysis was conducted and reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [Moher et al., 2009]. Objectives, eligibility criteria, and methods of analysis were specified in advance and published in a priori protocol (PROSPERO (CRD42022354454)) [Eskandari et al., 2022].

Search strategy and selection of studies

The search strategy was developed by two review authors (F.E. and E.A.K.). Search terms included a combination of key concepts in the research question, such as fluoride exposure, fluoride intake, fluoride biomarkers, spot urine, and nail clippings. We searched electronic databases (Medline, CINAHL, Web of Science, Scopus, ScienceDirect, Sage Journals Online, Campbell Collaboration, Cochrane Collaboration, and Embase); search engines (Google and Google Scholar); and Grey literature (OpenGrey, NICE Evidence Search, the Grey Literature Report, Bielefeld Academic Search Engine (BASE), and Australian Bureau of Statistics (ABS)). A detailed search strategy used for searching the databases is presented in Supplementary File 1.

The reference lists of eligible articles were also searched for relevant studies. The search for eligible papers was undertaken from 20th May 2021 to 22nd September 2022. The first author (F.E.) performed the searches and imported citations into an Endnote library for reference management. The citations were then exported into Covidence for screening. Duplicates were checked and removed in the Endnote and Covidence software.

All titles and abstracts were screened by F.E. and E.A.K. The full texts of seemingly eligible articles were also screened by two independent reviewers (F.E. and E.A.K.). Disagreements between reviewers were resolved through consensus or in consultation with another reviewer (F.V.Z. or L.A.).

Eligibility criteria

The inclusion and exclusion criteria were developed based on the population, exposure of interest, and outcomes (PEO) criteria.

Inclusion criteria

Participants

We considered studies involving humans as participants for inclusion in this review. Human participants comprised of children and/or adults of any age, gender, or ethnicity.

Exposure

The review included studies that have examined all forms of exposure to fluoride, such as water, diet, unintentional ingestion of dental products (e.g., toothpaste, mouth-rinses, fluoride-varnish), and air through experimental and environmental means.

Outcomes

We included studies that assessed the use of spot urine and/or nail clippings to monitor fluoride intake/exposure. Studies also had to report the correlation between fluoride exposure and fluoride concentrations of spot urine and/or nail clippings.

Study types

All experimental and observational research studies were considered for inclusion in this review. This included but was not limited to, randomised controlled studies, cohort studies with measurements made at a single time point (cross-sectional), pre-post studies, and other longitudinal studies measuring data at multiple time points.

Data extraction

A standardised data extraction form was developed in the Covidence software to extract relevant information from included studies. Specific information that was extracted included the year of publication, title, aim/objective of the study, study design, country, setting, population demographics,

exposure/intake data, methods of data collection, analytical procedures, and outcome(s) of interest to the review questions.

The data extraction form was first pilot-tested on 10% of the included articles before commencing data extraction. Data extraction was undertaken by one reviewer (F.E. or S.J.) and verified by another (E.A.K), using the Covidence software.

Assessment of methodological quality

Two reviewers (F.E and E.A.K) independently conducted a quality assessment of each included study using the Mixed-Methods Appraisal (MMA) tool developed by Hong et al. [Hong et al., 2018]. The MMA tool is useful for assessing the quality of studies in reviews with heterogenous study designs and contains specific questions for different study designs [Hong et al., 2018]. Any disagreements between reviewers were resolved by consensus.

Meta-analysis methods

Meta-analyses were conducted to assess the correlations between fluoride intake and: 1) fluoride concentration of spot urine; 2) fluoride concentration of nail clippings (toenail or fingernail); 3) fluoride/creatinine ratio of spot urine; and 4) fluoride/specific gravity ratio of spot urine.

Analyses (1) and (2) were considered to be primary analyses, whereas analyses (3) and (4) were secondary analyses. All meta-analyses (primary and secondary) considered studies based on either: (i) studies conducted on adults (≥ 18 years) only; (ii) studies conducted on children (<18 years) only; (iii) studies conducted on mixed adult/children groups. Within each group of studies, some variation in the age range of subjects could exist. For analysis 2, further subgroup analyses were proposed based on the type of nail clippings: toenail and fingernail. Studies that provided aggregate data (e.g., community) level were not included in the meta-analyses.

Random effects meta-analyses were conducted using the DerSimionian and Laird estimation method [DerSimonian and Laird, 2015]. These models were chosen to reflect recognised clinical and methodological heterogeneity across included studies, such as the ages of the participants in each constituent study.

Unadjusted correlation coefficients were used in the meta-analyses to avoid the introduction of additional heterogeneity caused by variation in included controlling covariates. Where not reported directly, correlation coefficients were calculated from simple regression coefficients and/or t-statistics in conjunction with study sizes. Correlation coefficients were then transformed using the Fisher z-transformation (inverse hyperbolic tangent) for meta-analysis. Back transformations were used to

transform the resulting pooled estimate back to the original metric. Correlation coefficients from subgroups reported separately within a single study were combined into a single measure by averaging transformed values and applying a back-transformation to the averaged measure.

For the primary analyses, forest plots were conducted for meta-analyses of the correlation coefficients, reporting the synthesised estimates and associated 95% confidence interval (CI), and a Z-test for the estimated effect (i.e., the correlation between fluoride intake and biomarker). Heterogeneity statistics were also reported, including Cochran's Q test for heterogeneity, the I^2 statistic (proportion of variation across studies ascribed to heterogeneity), and the τ^2 statistic (an estimate of between-study variance).

Sensitivity analyses were conducted on the primary meta-analyses to assess the robustness of the derived estimates. Each of the included studies was omitted in turn, and a meta-analysis was conducted based on the remaining studies, with results plotted on an influence plot. Any study which was suspected of excessive influence on the resulting influence plot (considered to be indicated by the point estimate of the "omitted" analysis of a study lying outside the CI of the "combined" analysis) was flagged as an influential study.

Heterogeneity was further explored in the primary analyses with Galbraith plots (plots of a standardized effect against the reciprocal of the standard error of the effect) of meta-analyses of primary outcomes. In the absence of substantial heterogeneity, it is to be expected that around 95% of included studies will lie within the shaded area of the plot (95% CI region). Imprecise estimates of effect lie near the origin, and precise estimates are further away.

Key findings from subgroup and secondary analyses, were tabulated without graphical representation, including the synthesized effect and 95% CI, heterogeneity as measured by the I^2 statistic and the result of the Z-test for effect. Between-group effects were also calculated where possible for subgroup analyses.

Results

Search results

The initial search results yielded 15,177 articles (10,121 after the removal of duplicates). These were then screened by title and abstract independently by two authors (F.E. and E.A.K.) to identify those potentially meeting the inclusion criteria. Following title and abstract screening, 9753 articles were excluded resulting in 368 articles assessed at the full-text screening stage. The full-text screening was conducted by three review authors (F.E., E.A.K., and S.J.), and disagreements between reviewers were resolved through consensus or by another reviewer (F.V.Z.). After the full-text screening, 321 articles

were excluded, including 44 articles in the systematic review. The PRISMA flow diagram shows the number of articles at each stage (Fig 1).

The detailed study characteristics and outcomes are presented in Supplementary File 2 and Supplementary File 3.

Study characteristics

Overall, the included studies originated from 21 countries across various continents (Supplementary File 2). Nine studies were conducted in China, seven in India, four in Brazil, three in Canada, two in the UK, two in Mexico, two in Nigeria, two in Ethiopia, one in Japan, one in Germany, one in the USA, one in Serbia, one in Hungary, one in Portugal, one in Poland, one in Slovakia, one in Tanzania, and one in Jamaica. The remaining three studies were jointly conducted in 'Mexico and Canada', 'Belgium and France', and 'Brazil and Peru'.

A summary of the characteristics of the included studies in the systematic review is presented in Table 1. Of the included studies, 70.5% were published after 2014, 77.3% had a cross-sectional design, 59.1% evaluated spot urine as a biomarker for fluoride exposure, and 59.1% were in children.

Methodological assessment

Based on the MMA tool [Hong et al., 2018], the methodological quality of the included studies was assessed using criteria/items specific to quantitative randomised controlled trials (including cohort studies, cross-sectional studies, before and after studies) and quantitative descriptive studies (including longitudinal studies). As shown in Figure 2, fourteen articles (31.8%) met all quality assessment criteria, and twenty-three studies (52.3%) met six out of the seven assessment criteria. Six studies (13.6%) met five out of the seven criteria, while one study (2.3%) met four of the assessment criteria. Most studies were therefore deemed to be of very good quality, with the risk of selection bias remaining low. Common issues with quantitative non-randomised studies were a lack of information about confounders.

Meta-analysis 1: Correlation between fluoride intake and fluoride concentration of spot urine

Twenty-five studies that either directly or indirectly reported the correlation between fluoride intake and fluoride concentration of spot urine in children, adults, and mixed groups of children and adults in non-aggregated data were included in this meta-analysis. The meta-analysis revealed a synthesised estimate of the Fisher-transformed correlation coefficient of 0.674 (95% confidence interval 0.623 to 0.725). This corresponded to a synthesised estimate of the back-transformed correlation coefficient of 0.588 (95% CI 0.553 to 0.620). A *Z*-test of the standardised mean effect revealed strong evidence (at

the 5% significance level) for a non-zero effect (Z=26.1; p<0.001). Individual estimates for the back-transformed correlation coefficient ranged from 0.310 [Heintze et al., 1998] to 0.995 [Saxena et al., 2012].

Cochran's χ^2 test for heterogeneity revealed strong evidence (at the 5% significance level) for statistical heterogeneity ($\chi^2_{(24)}$=1979; p<0.001). The I^2 statistic was revealed to be 98.8%, indicating a very high proportion of variation across studies ascribed to heterogeneity. The data is summarised in a forest plot (Fig 3).

A sensitivity analysis revealed that the results of Saxena et al. (2012) [Saxena et al., 2012] were exerting excessive influence on the overall effect, with the point estimates of the omitted analysis lying outside the 95% CI associated with the estimate of the combined analysis (Supplementary File 4, Fig 1).

A meta-analysis of all included studies except the study of Saxena et al. (2012) [Saxena et al., 2012] revealed that a synthesised estimate of the Fisher-transformed correlation coefficient was 0.569 (95% CI 0.531 to 0.608). This corresponded to a synthesised estimate of the back-transformed correlation coefficient of 0.503 (95% CI 0.486 to 0.543). A Z-test of the standardised mean effect revealed strong evidence (at the 5% significance level) for a non-zero effect (Z=29.1; p<0.001). Hence the exclusion of the study of Saxena et al. resulted in a reduction of the synthesised estimate of the correlation coefficient of about 14%.

The exclusion of the study of Saxena et al. (2012) [Saxena et al., 2012] had no substantive effect on inferences of study heterogeneity as Cochran's χ^2 test for heterogeneity revealed strong evidence (at the 5% significance level) for statistical heterogeneity ($\chi^2_{(23)}$ = 898; p<0.001). The I^2 statistic was revealed to be 97.4%, indicating a high proportion of variation across studies ascribed to heterogeneity.

Meta-analysis 2: Correlation between fluoride intake and fingernail/toenail fluoride concentrations

Eleven studies that either directly or indirectly reported this correlation in non-aggregated data were included in the meta-analysis of the correlation between fluoride intake and fluoride concentrations of fingernails and toenails.

The synthesised estimate of the Fisher-transformed correlation coefficient was 0.938 (95% CI 0.520 to 1.355). This corresponded to a synthesised estimate of the back-transformed correlation coefficient of 0.734 (95% confidence interval 0.478 to 0.875). A Z-test of the standardised mean effect revealed strong evidence (at the 5% significance level) for a non-zero effect (Z=4.40; p<0.001). Individual estimates for the back-transformed correlation coefficient ranged from -0.281 [Sousa et al., 2018] to 0.977 [Vidyadharan et al., 2020].

Cochran's χ^2 test for heterogeneity revealed strong evidence (at the 5% significance level) for statistical heterogeneity ($\chi^2_{(10)}$=480; p<0.001) and I^2 statistic was 97.9%, indicating a high proportion of variation across studies. The data is summarised in a forest plot (Fig 4).

A sensitivity analysis revealed that none of the included studies was exerting excessive influence on the analysis, with all point estimates of the omitted analyses lying within the 95% CI associated with the estimate of the combined analysis. Estimates and associated CIs are plotted on an influence plot (Supplementary File 4, Fig 2).

Subgroup analyses

Tables 2–4 summarise the findings of the primary and secondary subgroup meta-analyses conducted in cases where two or more constituent studies could be identified.

The findings of subgroup meta-analyses showed a moderately-strong positive significant estimate of the effect of the correlation between fluoride intake and fluoride concentration of spot urine for children only (Table 2). The corresponding estimate of effect was moderate for adults only and mixed adults and children, although the effect was not statistically significant for the mixed group (Table 2).

A moderately strong positive effect size was also found for the correlation between fluoride intake and nail clippings fluoride concentrations for all three categories of age groups. However, the estimated effect was not statistically significant for the correlation between fluoride intake and fingernail fluoride concentration for children only and adults only but for the mixed group (Table 3).

The findings of secondary meta-analyses showed a strong positive significant effect size for the correlation between fluoride intake and fluoride/creatinine ratio of spot urine for children (Table 4). However, a moderately weak effect size was found for the correlation between fluoride intake and the fluoride/specific gravity ratio of spot urine in adults.

Discussion

This systematic review and meta-analysis explored the relationship between fluoride intake and fluoride concentration of spot urine and nail clippings (i.e., fluoride biomarkers). The included studies were predominantly of high quality (86% of included studies). Results from the meta-analysis indicated a strong correlation between fluoride intake and fluoride concentration of nail clippings in all age groups. The fluoride concentration of spot urine, when normalised to urinary creatinine concentration, was also found to have a strong correlation with fluoride intake in children. However, the findings should be

taken with caution as most studies did not estimate total fluoride intake from all sources (e.g. diet, dentifrices), and some used the fluoride concentration of water as a proxy of fluoride intake.

Overall, 44 studies originating from 21 countries across various continents met the inclusion criteria and were included in this review. More than 70% of the included studies were conducted after the WHO publication [WHO, 2014] in 2014, and particularly in children (59%). This is mainly because dental fluorosis is an adverse effect of excessive fluoride intake during childhood, hence the need for a simple method for surveillance of fluoride exposure in this age group.

The results of our meta-analysis for 25 studies (Fig 3), which explored the overall correlation between fluoride intake and fluoride concentration of spot urine samples (normalised and un-normalised), revealed a positive moderate correlation (0.67) which was statistically significant ($p<0.001$). The narrow-observed CI (0.62, 0.72) also indicates a good level of precision. When we explored age groups in the subgroup analysis (Table 2), we found that the corresponding correlation was statistically significant in children only and adults only ($p<0.001$), but not in the mixed adults and children group ($p=0.143$). The between-group effect analysis also showed a statistically significant ($p<0.001$) difference between children and adults. This could be explained by the differences in fluoride metabolism between children and adults. Under normal conditions, almost 45% of the fluoride absorbed by healthy children is excreted in the urine, whereas the corresponding value is 60% for adults [Villa et al., 2010]. An analysis of available data for 212 children and 283 adults from different geographical areas showed a strong linear relationship between fluoride intake and 24-hour urinary fluoride excretion for both age groups but with different slopes for young children and adults [Villa et al., 2010]. The finding of our systematic review and the former study suggests that the correlation between fluoride intake and excretion should be investigated separately for different age groups.

There was also a very strong and significant positive correlation (0.94, 95% CI 0.52 to 1.36) between fluoride intake and fluoride concentration of nail clippings for the 11 studies which reported this correlation in non-aggregated data (Fig 4). The subgroup analysis based on the type of nail clippings (finger or toe) and age groups also showed a statistically significant strong correlation between fluoride intake and fingernail fluoride when all studies were combined (n=6) but not significant for children (n=3) and adults (n=2) investigated separately (Table 3). However, a moderate correlation was found between fluoride intake and toenail fluoride concentration, which was statistically significant when all studies were combined (n=6) as well as for children only (n=4). Due to the scarcity of relevant studies on these relationships, the findings should be interpreted with caution. A study with 89 children and their parents [Sah et al., 2020], in which total daily fluoride intake and fluoride concentrations of toe- and finger-nails were assessed, found no significant differences in fingernail fluoride in both children and parents but a statistically significant difference in toenail fluoride concentration in parents. This

study also found a statistically significant difference in toenail fluoride concentration in parents but not in children. A review of published studies [Pessan and Buzalaf, 2011] on the relationship between fluoride intake and nail fluoride concentration found higher fluoride concentrations in fingernails than in toenails in three out of the seven included studies. The higher fingernail fluoride concentration could be due to the higher vulnerability of fingernails to external fluoride contaminations (such as soil and nail varnishes), as well as higher blood supply in fingernails and consequently higher uptake of fluoride from plasma. Therefore, more epidemiological studies are needed to assess the sensitivity of nails as a biomarker of fluoride exposure in different age groups and populations with different lifestyles and behaviour. For instance, ingesting soil could be a major route through which young children are exposed to environmental pollutants (such as fluoride) as a result of their hand-to-mouth behaviour.

Additionally, the secondary meta-analyses (Table 4) showed a very strong correlation between fluoride intake and fluoride/creatinine ratio of spot urine in children (n=2) but a weak correlation between fluoride intake and fluoride/specific gravity ratio of spot urine in adults (n=2). The basis for creatinine and/or specific gravity adjustment of concentrations of biomarkers in spot urine samples is to compensate for variation in the urine dilution caused by differences among individuals in their fluid intake, physical activity, temperature, etc. Although both creatinine and specific gravity have been used for clinical diagnosis as well as clinical studies, it has been shown that urinary creatinine fluctuated more than specific gravity by age and gender [Suwazono et al., 2005]. Since a very small number of studies were identified and included in the sub-group analyses and none of these studies included both children and adults, drawing a firm conclusion on the reliability of these biomarkers may not be possible. Additionally, our findings showed overall high heterogeneity levels on the standardized and unstandardized meta-analysis for both spot urine (I^2=98.8%) and nail clippings (I^2=97.9%). Removing one outlier study [Saxena et al., 2012], resulted in a small reduction in the synthesised estimate of the correlation coefficient with no significant effect on inferences of study heterogeneity. The high heterogeneity levels of studies, in this review, could be due to variability across the study designs, fluoride measurement methods, participants' characteristics (age), and more importantly source of fluoride exposure, and methods of exposure assessments (e.g. using water as a proxy of fluoride exposure, duplicate-plate diet collection, food-diaries, etc). Although we carried out several subgroup analyses to help understand the effects of two broad age groups and types of biomarkers, we were unable to explore other key components, such as narrower age groups (e.g., younger- and older-children), type/source of fluoride exposure (e.g. water, diet, toothpaste ingestion) as well as the time of day samples were taken and the number of collected samples, due to a small (or no) relevant studies for such subgroup analyses. In the case of spot urine collection, it is recommended to take them several times within a day to reflect the variation in fluoride intake as well as the period that urine accumulated in the bladder – the shorter the accumulation period, the shorter-lived the peak level of fluoride concentration [WHO, 2014].

In general, the fluoride concentration of spot urine and nail clippings can be influenced by several environmental and biological factors. For instance, factors affecting urinary fluoride concentration include environmental temperature, degree of hydration, diet (plant- or meat-based diet), altitude of residence, certain diseases, and acid-base balance [Rugg-Gunn et al., 2011]. The factors influencing nail fluoride concentration include age, gender, geographical area, nail growth rate, length, and site of collection (thumb, toe, finger etc) [Pessan and Buzalaf, 2011].

Finally, it should be highlighted that statistical analysis of the relationships between fluoride exposure and fluoride biomarkers (such as urine) has clearly shown that the 95% prediction intervals linked with the regression line do not support biomarkers as an accurate estimator of fluoride exposure on an individual basis but only at population/group levels [Villa et al., 2010, Rugg-Gunn et al., 2011].

Strength and limitations

To our knowledge, this is the first systematic review and meta-analysis to quantitatively examine the association between fluoride intake and fluoride concentrations in spot urine and nail clippings as biomarkers of fluoride exposure.

This review employed a rigorous methodology, which included a comprehensive database search, yielding 15,117 studies with no restriction on year of publication. It included a large overall sample size (n=694,578 individuals), with no age restriction applied. All screening, data extraction, and quality assessments were conducted in duplicate using standardised protocols. Additionally, included studies represented a range from low- to high-income countries across various continents, and most studies (86%) were considered as having high quality.

However, it also contains some limitations, as only studies published in the English language were included and therefore relevant and important data from studies in other languages may have been missed. Likewise, the cross-sectional nature of most of the included studies might prevent drawing conclusions about the causality and direction of associations. However, the significant associations found, in this systematic review, between fluoride intake and fluoride concentration of spot urine and nail clippings are promising and could form a useful starting point for future research into the causal pathways between fluoride exposure and spot urine/nail clippings as fluoride biomarkers. A greater focus on longitudinal studies would be therefore highly encouraged.

Conclusions

Finding viable and accurate biomarkers for fluoride exposure has gained much attention over the past several decades due to the expansion in knowledge of fluoride metabolism, technical advances in fluoride measurements and the importance of fluoride surveillance in populations.

Spot urine and nail clippings have the potential to be employed as non-invasively obtained biomarkers in populations. This systematic review found fluoride concentrations in spot urine (when normalised to urinary creatinine excretion) and nail clippings were strongly correlated with fluoride intake in a group of children and adults. However, due to the shortage of related studies and the high heterogenicity of the included studies, more research is needed to establish the validity of these biomarkers. Future research should explore the cost-effectiveness and generalizability of these biomarkers for different fluoridation schemes. In particular, high-quality studies are needed to explore the different methods of fluoride delivery (e.g., dentifrice, fluoridated-salt or -water), different settings /geographical areas, and targeted populations.

Conflict of Interest: No conflicts of interest.

Funding: This research was funded by The Borrow Foundation [grant number NFB/KH]

Statement of Ethics: An ethics statement is not applicable because this study is based exclusively on published literature.

Author Contributions: F.V.Z. and L.A. conceptualised the study and formulated the research questions and contributed to developing the PROSPERO protocol. F.E. performed the searches. F.E. and E.A.K. conducted the title, abstract and full-text screens independently. F.E., E.A.K., and S.J. independently extracted data. F.E., and E.A.K conducted quality assessment. J.S. conducted meta-analysis. F.V.Z. and F.E. led the writing of the manuscript and all authors have commented on drafts of the manuscript. All authors read and approved the final manuscripts.

Data Availability: All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

References

Abduweli Uyghurturk D, Goin DE, Martinez-Mier EA, Woodruff TJ, DenBesten PK. Maternal and fetal exposures to fluoride during mid-gestation among pregnant women in northern California. Environ Health. 2020;19(1):38. https://doi:10.1186/s12940-020-00581-2

Ailani V, Gupta RC, Gupta SK, Gupta K. Oxidative stress in cases of chronic fluoride intoxication. Indian J Clin Biochem. 2009;24(4):426-29. https://doi:10.1007/s12291-009-0076-0

Antonijevic E, Mandinic Z, Curcic M, Djukic-Cosic D, Milicevic N, Ivanovic M, et al. "Borderline" fluorotic region in Serbia: correlations among fluoride in drinking water, biomarkers of exposure and dental fluorosis in schoolchildren. Environ Geochem Health. 2016;38(3):885-96. https://doi:10.1007/s10653-015-9769-x

Baez RJ, Petersen PE, Marthaler TM. Basic methods for assessment of renal fluoride excretion in community prevention programmes for oral health. Geneva: World Health Organization, 2014. 96 p. https://apps.who.int/iris/handle/10665/112662

Bhattacharya P, Adhikari S, Samal AC, Das R, Dey D, Deb A, et al. Health risk assessment of co-occurrence of toxic fluoride and arsenic in groundwater of Dharmanagar region, North Tripura (India). Groundw Sustain Dev. 2020;11. https://doi.org/10.1016/j.gsd.2020.100430

Buzalaf MAR, Rodrigues MHC, Pessan JP, Leite AL, Arana A, Villena RS, et al. Biomarkers of fluoride in children exposed to different sources of systemic fluoride. J Dent Res. 2011;90(2):215-19. https://doi:10.1177/0022034510385937

Cardenas-Gonzalez M, Osorio-Yanez C, Gaspar-Ramirez O, Pavkovic M, Ochoa-Martinez A, Lopez-Ventura D, et al. Environmental exposure to arsenic and chromium in children is associated with kidney injury molecule-1. Environ Res. 2016 Oct;150:653-62. https://doi:10.1016/j.envres.2016.06.032

Choi AL, Zhang Y, Sun G, Bellinger DC, Wang K, Yang XJ, et al. Association of lifetime exposure to fluoride and cognitive functions in Chinese children: a pilot study. Neurotoxicol Teratol. 2015;47:96-101. https://doi:10.1016/j.ntt.2014.11.001

Cunningham JEA, McCague H, Malin AJ, Flora D, Till C. Fluoride exposure and duration and quality of sleep in a Canadian population-based sample. Environ Health. 2021;20(1):16. https://doi:10.1186/s12940-021-00700-7

448 de Almeida BS, da Silva Cardoso VE, Buzalaf MA. Fluoride ingestion from toothpaste and diet in 1-
449 to 3-year-old Brazilian children. Community Dent Oral Epidemiol. 2007;35(1):53-63.
450 https://doi:10.1111/j.1600-0528.2007.00328.x

451 DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. Contemp Clin Trials. 2015
452 Nov;45(Pt A):139-45. doi:10.1016/j.cct.2015.09.002

453 Eskandari F, Kumah E, John S, Azevedo L, Stephenson J, Zohoori F. A systematic review and meta-
454 analysis of fluoride exposure in community prevention programmes for oral health using nail
455 clippings and spot urine samples 2022. PROSPERO 2022 CRD42022354454 Available from:
456 https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022354454

457 Fukushima R, Rigolizzo DS, Maia LP, Sampaio FC, Lauris JRP, Buzalaf MAR. Environmental and
458 individual factors associated with nail fluoride concentration. Caries Res. 2009;43(2):147-54.
459 https://doi:10.1159/000211718

460 Green R, Lanphear B, Hornung R, Flora D, Martinez-Mier EA, Neufeld R, et al. Association Between
461 Maternal Fluoride Exposure During Pregnancy and IQ Scores in Offspring in Canada. JAMA Pediatr.
462 2019;173(10): 940–48. https://doi:10.1001/jamapediatrics.2019.1729

463 Green R, Till C, Cantoral A, Lanphear B, Martinez-Mier EA, Ayotte P, et al. Associations between
464 Urinary, Dietary, and Water Fluoride Concentrations among Children in Mexico and Canada. Toxics.
465 2020;8(4):110. https://doi:10.3390/toxics8040110

466 Haufroid V, Gardinal S, Licot C, Villalpando ML, Obbergh LV, Clippe A, et al. Biological
467 monitoring of exposure to sevoflurane in operating room personnel by the measurement of
468 hexafluoroisopropanol and fluoride in urine. Biomarkers. 2000;5(2):141-51.
469 https://doi:10.1080/135475000230451

470 Heintze SD, Bastos JR, Bastos R. Urinary fluoride levels and prevalence of dental fluorosis in three
471 Brazilian cities with different fluoride concentrations in the drinking water. Community Dent Oral
472 Epidemiol. 1998;26(5):316-23. https://doi: 10.1111/j.1600-0528.1998.tb01967.x

473 Hong QN, Pluye P, Fàbregues S, Bartlett G, Boardman F, Cargo M, et al. Mixed Methods Appraisal
474 Tool (MMAT) Version 2018 User Guide. 2018. Available from
475 http://mixedmethodsappraisaltoolpublic.pbworks.com/w/file/fetch/127916259/MMAT_2018_criteria-
476 manual_2018-08-01_ENG.pdf.

Idowu OS, Duckworth RM, Valentine RA, Zohoori FV. Biomarkers for the Assessment of Fluoride Exposure in Children. Caries Res. 2020;54(2):134-43. https://doi:10.1159/000504166

Idowu OS, Duckworth RM, Valentine RA, Zohoori FV. Biomarkers for the Assessment of Exposure to Fluoride in Adults. Caries Res. 2021a;55(4):292-300. Idowu OS, Duckworth RM, Valentine RA, Zohoori FV. Biomarkers for the Assessment of Exposure to Fluoride in Adults. Caries Res. 2021b;55(4):292-300.

Jiménez-Córdova MI, González-Horta C, Ayllón-Vergara JC, Arreola-Mendoza L, Aguilar-Madrid G, Villareal-Vega EE, et al. Evaluation of vascular and kidney injury biomarkers in Mexican children exposed to inorganic fluoride. Environmental Res. 2019;169:220-28. https://doi:10.1016/j.envres.2018.10.028

Kertész P, Bánóczy J, Ritlop B, Bródy A, Péter M. The determination of urinary fluoride/creatinine ratio (Q) in monitoring fluoride intake. Acta Physiol Hung. 1989;74(3-4):209-14.

Kumah EA, Eskandari F, Azevedo LB, John S, Zohoori FV. Mapping the evidence for monitoring fluoride exposure in community prevention programmes for oral health using nail clippings and spot urine samples: a scoping review. BMC Oral Health. 2022;22(1):1-11. doi:10.1186/s12903-022-02615-2

Lavalle-Carrasco J, Molina-Frechero N, Nevarez-Rascon M, Sanchez-Perez L, Hamdan-Partida A, Gonzalez-Gonzalez R, et al. Recent biomarkers for monitoring the systemic fluoride levels in exposed populations: A systematic review. Int J Environ Res Public Health. 2021 01 Jan;18(1):1-14. https://doi:10.3390/ijerph18010317

Linhares DPS, Garcia PV, Amaral L, Ferreira T, Cury JA, Vieira W, et al. Sensitivity of two biomarkers for biomonitoring exposure to fluoride in children and women: A study in a volcanic area. Chemosphere. 2016;155:614-20. https://doi:10.1016/j.chemosphere.2016.04.092

Liu L, Wang M, Li Y, Liu H, Hou C, Zeng Q, et al. Low-to-moderate fluoride exposure in relation to overweight and obesity among school-age children in China. Ecotoxicol Environ Saf. 2019;183:109558. https://doi:10.1016/j.ecoenv.2019.109558

Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097

506 Mondal D, Dutta G, Gupta S. Inferring the fluoride hydrogeochemistry and effect of consuming
507 fluoride-contaminated drinking water on human health in some endemic areas of Birbhum district,
508 West Bengal. Environ Geochem Health. 2016;38(2):557-76. https://doi:10.1007/s10653-015-9743-7

509 O'Mullane DM, Baez RJ, Jones S, Lennon MA, Petersen PE, Rugg-Gunn AJ, et al. Fluoride and Oral
510 Health. Community Dent Health. 2016 Jun;33(2):69-99.

511 Opydo-Szymaczek J, Ogińska M, Wyrwas B. Fluoride exposure and factors affecting dental caries in
512 preschool children living in two areas with different natural levels of fluorides. J Trace Elem Med
513 Biol. 2021;65:126726. https://doi:10.1016/j.jtemb.2021.126726

514 Pessan JP, Buzalaf MRA. Historical and Recent Biological Markers of Exposure to Fluoride. Monogr
515 Oral Sci. 22 (2011) 52–65. https:// doi:10.1159/000325145.

516 Rango T, Vengosh A, Jeuland M, Tekle-Haimanot R, Weinthal E, Kravchenko J, et al. Fluoride
517 exposure from groundwater as reflected by urinary fluoride and children's dental fluorosis in the Main
518 Ethiopian Rift Valley. Sci Total Environ. 2014;496:188-97.
519 https://doi:10.1016/j.scitotenv.2014.07.048

520 Rango T, Vengosh A, Jeuland M, Whitford GM, Tekle-Haimanot R. Biomarkers of chronic fluoride
521 exposure in groundwater in a highly exposed population. Sci Total Environ. 2017;596-597:1-11.
522 doi:10.1016/j.scitotenv.2017.04.021

523 Rugg-Gunn AJ, Villa AE, Buzalaf MRA. Contemporary biological markers of exposure to fluoride.
524 Monogr Oral Sci. 2011;22:37-51. doi:10.1159/000325137Sah

525 O, Maguire A, Zohoori FV. Effect of altitude on urinary, plasma and nail fluoride levels in children
526 and adults in Nepal. J Trace Elem Med Biol. 2020 Sep;57:1-8.
527 https://doi:10.1016/j.jtemb.2019.09.003

528 Saxena S, Sahay A, Goel P. Effect of fluoride exposure on the intelligence of school children in
529 Madhya Pradesh, India. J Neurosci Rural Pract. 2012;3(2):144-49. https://doi:10.4103/0976-
530 3147.98213

531 Schwarz M, Salva J, Vanek M, Rasulov O, Darmová I. Fluoride Exposure and the Effect of Tobacco
532 Smoking on Urinary Fluoride Levels in Primary Aluminum Workers. Appl Sci. 2020;11(1).
533 https://doi.org/10.3390/app11010156

534 Singh N, Verma KG, Verma P, Sidhu GK, Sachdeva S. A comparative study of fluoride ingestion
535 levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school
536 children from endemic and non-endemic fluorosis areas. SpringerPlus. 2014;3:7.
537 https://doi:10.1186/2193-1801-3-7

538 Sousa ETd, Alves VF, Maia FBM, Nobre-Dos-Santos M, Forte FDS, Sampaio FC. Influence of
539 Fluoridated Groundwater and 1,100 Ppm Fluoride Dentifrice on Biomarkers of Exposure to Fluoride.
540 Braz Dent J. 2018;29(5):475-82. https://doi:10.1590/0103-6440201801959

541 Suwazono Y, Åkesson A, Alfvén T, Järup L, Vahter M. Creatinine versus specific gravity-adjusted
542 urinary cadmium concentrations. Biomarkers. 2005;10(2-3):117-26.
543 https://doi:10.1080/13547500500159001

544 Till C, Green R, Grundy JG, Hornung R, Neufeld R, Martinez-Mier EA, et al. Community Water
545 Fluoridation and Urinary Fluoride Concentrations in a National Sample of Pregnant Women in
546 Canada. Environ Health Perspect. 2018 Oct;126(10):107001. doi:10.1289/EHP3546

547 Vidyadharan M, Issac JS, Joseph AM, Joseph A, John D, Varadharaju VK. Comparative Evaluation
548 of Hair, Fingernails, and Toenails as Biomarkers of Fluoride Exposure: A Cross-Sectional Study. J Int
549 Soc Prev Community Dent. 2020;10(3):269-78. https://doi:10.4103/jispcd.JISPCD_52_20

550 Villa A, Anabalon M, Zohouri V, Maguire A, Franco AM, Rugg-Gunn A. Relationships between
551 fluoride intake, urinary fluoride excretion and fluoride retention in children and adults: an analysis of
552 available data. Caries Res. 2010;44(1):60-8. https:// doi:10.1159/000279325

553 Wang C, Gao Y, Wang W, Zhao L, Zhang W, Han H, et al. A national cross-sectional study on effects
554 of fluoride-safe water supply on the prevalence of fluorosis in China. BMJ Open. 2012;2(5) e001564.
555 https://doi:10.1136/bmjopen-2012- 001564

556 Wang M, Liu L, Li H, Li Y, Liu H, Hou C, et al. Thyroid function, intelligence, and low-moderate
557 fluoride exposure among Chinese school-age children. Environ Int. 2020;134:105229.
558 doi:10.1016/j.envint.2019.105229

559 Warpeha RA, Marthaler TM. Urinary fluoride excretion in Jamaica in relation to fluoridated salt.
560 Caries Res. 1995;29(1):35-41. https://doi:10.1159/000262037

561 Watanabe M, Kono K, Orita Y, Dote T, Usuda K, Takahashi Y, et al. Influence of dietary fluoride
562 intake on urinary fluoride concentration and evaluation of corrected levels in spot urine. Fluoride.
563 1995;28(2):61-70.

Wu JH, Qin M, Li DD, Yang D, Li BY, Liu XN, et al. Correlation analysis of urinary fluoride levels and the daily intake of fluoride in brick tea type fluorosis areas. Fluoride. 2016;49(4):449-57.

Yadav JP, Lata S. Urinary fluoride levels and prevalence of dental fluorosis in children of Jhajjar District, Haryana. Indian J Med Sc. 2003;57(9):394-99.

Yoder KM, Mabelya L, Robison VA, Dunipace AJ, Brizendine EJ, Stookey GK. Severe dental fluorosis in a Tanzanian population consuming water with negligible fluoride concentration. Community Dent Oral. 1998;26(6):382-93. https://doi:10.1111/j.1600-0528.1998.tb01976.x

Yu X, Chen J, Li Y, Liu H, Hou C, Zeng Q, et al. Threshold effects of moderately excessive fluoride exposure on children's health: A potential association between dental fluorosis and loss of excellent intelligence. Environ Int. 2018;118:116-24. https://doi:10.1016/j.envint.2018.05.042

Zhang S, Zhang X, Liu H, Qu W, Guan Z, Zeng Q, et al. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. Toxicol Sci. 2015;144(2):238-45. https://doi.org/10.1093/toxsci/kfu311

Zhou G, Yang L, Luo C, Liu H, Li P, Cui Y, et al. Low-to-moderate fluoride exposure, relative mitochondrial DNA levels, and dental fluorosis in Chinese children. Environ Int. 2019;127:70-77. https://doi:10.1016/j.envint.2019.03.033

Zhou G, Zhao Q, Luo C, Liu H, Li P, Cui Y, et al. Low-moderate fluoride exposure and intelligence among Chinese school-aged children: Role of circulating mtDNA content. Sci Total Environ. 2021;786. https://https://doi.org/10.1016/j.scitotenv.2021.147330

Zober A, Geldmacher von Mallinckrodt M, Schaller KH. Renal fluoride excretion as a useful parameter for monitoring hydrofluoric acid-exposed persons. Int Arch Occup Environ Health. 1977 Oct 17;40(1):13-24. doi:10.1007/BF00435513

Zohoori FV, Duckworth RM. Chapter 5: Microelements: Part II: F, Al, Mo and Co. Monogr Oral Sci. 2020;28:48-58. doi:10.1159/000455370 Zohoori FV, Maguire A. Determining an upper reference value for the urinary fluoride-creatinine ratio in healthy children younger than 7 years. Caries Res. 2017;51(4):283-89. https://doi:10.1159/000472263

Zohouri FV, Swinbank CM, Maguire A, Moynihan PJ. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? Community Dent Oral Epidemiol. 2006;34(2):130-38. doi:10.1111/j.1600-0528.2006.00269.x

Tables:

Table 1. Summary of characteristics of included studies

Table 2. Subgroup analysis* of effect estimates of correlation between fluoride intake and fluoride concentration of spot urine

Table 3. Subgroup analysis of effect estimates of correlation between fluoride intake and fluoride concentration of nail clippings

Table 4. Secondary outcomes of effect estimates of correlation between fluoride intake and fluoride/creatinine ratio of spot urine, as well as fluoride/specific gravity ratio of spot urine, respectively

Figure captions

Figure 1. Flow diagram outlining the study selection process (adapted from Moher et al [11]). (n=number of studies)

Figure 2. Quality assessment scores for included articles using the Mixed Methods Assessment Tool

Figure 3. Forest plot for meta-analysis of correlation between fluoride intake and fluoride concentration of spot urine

Figure 4. Forest plot for meta-analysis of correlation between fluoride intake and fingernail/toenail fluoride concentrations