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Fractional urinary fluoride excretion and nail fluoride concentrations in normal, wasted and stunted 4–5 year-old children in Nepal

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ABSTRACT

Introduction: It has been suggested that undernourished children are more likely to develop dental fluorosis. We investigated the effects of nutritional status on systemic fluoride metabolism including the proportion of ingested fluoride excreted through urine (i.e. fractional urinary fluoride excretion - FUFE) and fluoride concentration in nail clippings in children, aged 4−5 years, in Nepal.

Methods: Nutritional status was evaluated using weight-for-age (wasting) and height-for-age (stunting) indices. Total daily fluoride intake (TDFI) was estimated from diet and toothpaste ingestion and 24 -h urine collected to assess daily urinary fluoride excretion (DUFE). FUFE was calculated by dividing DUFE by TDFI. Nail clippings (finger and toe) were collected and analysed for fluoride concentration.

Results: Of the 100 children who participated, 89 provided information to assess FUFE and 51 children provided nail samples. Overall, 86.5 % of the 89 children were wasted and 39.3 % were stunted.

When the samples were pooled into binary (affected and non-affected) categories, mean TDFI and mean DUFE were statistically significantly higher in the 77 wasted children (57.7 and 29.7 μg/kgbw/d, respectively) than the 12 non-wasted children (39.4 and 17.0 μg/kgbw/d, respectively). TDFI and DUFE were also statistically significantly higher in the 35 stunted children (65.1 and 34.5 μg/kgbw/d, respectively) than in the 54 non-stunted children (48.8 and 23.7 μg/kgbw/d, respectively). However, mean FUFE was similar in all groups. There were no statistically significant differences in fluoride concentration of either fingernails or toenails among the different categories of wasting, while mean fingernail fluoride concentration was statistically significantly higher in stunted (5.4 μg/g) than in non-stunted children (3.5 μg/g).

Conclusion: Our study found no significant effect of nutritional status on the proportion of ingested fluoride excreted in urine (and consequently the proportion retained in the body). These findings suggest that nutritional status may be less likely to be a main risk factor for the development of dental fluorosis than children's dietary habits or total fluoride intake.

1. Introduction

Fluoride, a widely distributed trace element in the environment, plays an important role in the prevention of dental caries. However excessive exposure to fluoride during the periods when teeth are forming can result in dental fluorosis. The critical period for development of fluorosis in the permanent anterior teeth is the first five years of life [1]. Fluoride is also one of only few known agents that can stimulate bone cell proliferation and increase mineral deposition in cancellous bone. The concentration of fluoride in bone increases with the duration of exposure [2]. Chronic excessive exposure to fluoride during periods of bone modelling (growth) and/or remodelling could result in skeletal fluorosis [3].

Body fluoride burden from total fluoride exposure and/or body fluoride retention have been suggested as the true risk factors for fluorosis [4,5]. The main sources of fluoride ingestion are diet, unintentionally swallowed dental products (e.g. toothpaste) and fluoride supplements. In children, almost 35 % of ingested fluoride is excreted in the urine which is the main route for removal of fluoride from the body [6]. Body retained fluoride becomes mainly incorporated into calcified tissues including teeth. The rate of fluoride ingestion, excretion and consequently retention can be influenced by not only total fluoride

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intake but also other factors including nutritional status [7]. Reports from Saudi Arabia [8], East Africa [9], India [10] and Mexico [11] have indicated that undernourished children are more likely to develop moderate to severe dental fluorosis (Thylstrup and Fejerskov Index (TFI) ≥ 4).

Undernutrition continues to be a public health problem among children under the age of five years in developing countries. In children, the major indicators of nutritional status are stunting (low height-for-age), wasting (low weight-for-height) and being underweight (low weight-for-age) [12]. While stunting and wasting indicate chronic/recurrent and acute undernutrition respectively, being underweight is a composite indicator and includes both wasting and stunting. According to the UNICEF / WHO / World Bank Group Joint Child Malnutrition Estimates, globally 155 million children under five years of age are stunted and 52 million are wasted [13].

The literature is sparse in terms of the effect of nutritional status on systemic fluoride metabolism in young children. In 2016 the World Bank reported the prevalence of wasting and stunting in Nepalese children <5 years old as 11 % and 36 % respectively [14]. As part of a larger cross-sectional observational study [15], we aimed to investigate the effects of nutritional status on fractional urinary fluoride excretion (i.e the % of ingested fluoride which is excreted through urine) and fluoride concentration in finger- and toe-nails in children aged 4−5 years in Nepal. The objectives were to quantify total daily fluoride intake and urinary fluoride excretion in these children.

2. Materials and methods

The participants included in this current report were 4−5 year-olds, who had lived in one of the two municipalities in Nepal: Banepa in the Hill region and Rajbiraj in the Terai region with altitudes of 1,487 m and 76 m above sea level, respectively. Fluoride concentrations of drinking water in both areas were less than 0.4 mg/l [15].

The study was conducted according to guidelines laid down in Declaration of Helsinki and all procedures involving human subjects were approved by the School of Health & Social Care (SHSC) Research Governance and Ethics Committee in the UK (No. 077/13) and the Nepal Health Research Council (No. 121/13) in Nepal. An informed written parental consent was obtained for all participants.

2.1. Collection of data and samples

Information on fluoride exposure and samples of 24 h urine and nail clippings (finger- and toe-nails) were collected during three home visits, details of which are provided in our previous paper [15]. In summary, we measured the child's weight without shoes and jacket using a portable mechanical personal scale (BR2017; Camry, China) and the height using a portable stadiometer (DE56618903; ADE, Germany) without shoes. Information about each child's tooth-brushing habits was collected using a questionnaire and an expectorated sample from tooth-brushing (toothpaste and saliva) obtained during a tooth-brushing session to estimate fluoride intake from toothpaste ingestion. A three-day food diary with a post-completion interview was used to collect dietary information and parents were asked to collect their child's urine samples over a 24 h period and clip their child finger- and toe-nails during the same period. Samples of consumed food and drinks (including water) as well as toothpaste brands used by children were obtained and analysed for fluoride content.

2.2. Sample analysis

Details of sample preparation and analysis are provided elsewhere [15]. Samples of water and urine were measured directly in triplicate at room temperature using a fluoride ion-selective electrode and meter (Thermo Scientific Orion Star A214 Benchtop pH/ISE Meter) after adding TISAB III (total ionic strength adjustment buffer), while samples of food, toothpaste, tooth-brushing expectorate and nail were analysed indirectly after overnight hexamethyldisiloxane-acid diffusion [16].

2.3. Data preparation

Daily fluoride intake from toothpaste ingestion (μg/d) for each child was estimated by subtracting the total amount of fluoride in expectorated saliva from the total amount of fluoride in toothpaste dispensed on the toothbrush, multiplied by the corresponding frequency of daily brushing.

Daily fluoride intake from diet (μg/d) was calculated by multiplying the amount of each food and drink item consumed by the child (g/d) by its fluoride concentration (μg/g). Since no children used fluoride supplements, total daily fluoride intake (TDFI, μg/d) was calculated by adding the fluoride intake from diet and toothpaste ingestion.

The completeness of 24 h urine samples was checked by comparing urinary flow rate (ml/h) with the World Health Organization [17] criteria for clean urinary data for children aged 2–6 years: i.e. lower limits of 5 and 7 mL/h for children aged 2–4 and 4–6 years, respectively. Daily urinary fluoride excretion (DUFE, μg/d) was estimated by multiplying the volume by the fluoride concentration of the 24 h urine sample.

TDFI and DUFE were also calculated based on body weight (μg/kgbw/d) by dividing these values (μg/d) by body weight (kg).

Fractional Urinary Fluoride Excretion (FUFE %) for each child was calculated by dividing DUFE by TDFI and then multiplying by 100.

2.4. Characterisation of nutritional status

We used anthropometric data (age, weight and height) to calculate wasting (weight-for-height) and stunting (height-for-age):

Weight-for-height (%) = (weight of the child (kg)) / (weight of a normal child of the same height (kg)) × 100 %

Height-for-age (%) = (height of the child (m)) / (height of a normal child of the same age (m)) × 100 %

Wasting and stunting were then categorised based on Waterloo classifications [18,19]:

Wasting: none (>90 %), mild (90−81%), moderate (80−71%) and severe (<70 %).

Stunting: none (>95 %), mild (95−90%), moderate (89−85%) and severe (<85 %).

2.5. Statistical analysis

The data were analysed using SPSS (IBM SPSS Statistics, version 22). Mean differences (along with the 95 % confidence intervals) among all categories of wasting and stunting were quantified using a general linear model (GLM) with statistical significance set at $\alpha < 0.05$. The means for each group were adjusted for any influence of altitude by including altitude as a covariate.

Categories of mild, moderate and severe wasting as well as stunting were pooled together to represent two groups; all wasted and all stunted children. We then ran a between-subjects GLM which included a covariate of altitude to compare means for each parameter between the non-stunted and stunted children as well as between the non-wasted and wasted children.

3. Results

In total, 100 children took part in the study, of whom 89 completed the three-day food diary and provided 24 h urine samples as well as expectorated toothbrushing samples. In addition, 51 children provided finger- and/or toe-nail samples.

Table 1 presents the mean (SD) age (yr), weight (kg), height (cm),

Table 1
Anthropometric characteristics of children who took part in the study.

Anthropometric characteristics	All [n = 100]	Completers [n = 89]	Withdrawers [n = 11]
Age (y)	4.6 (0.5)	4.6 (0.5)	4.3 (0.5)
Weight (kg)	15.7 (2.4)	15.9 (2.4)	13.8 (1.6)
Height (cm)	100.4 (7.5)	100.7 (7.5)	98.0 (8.1)
Weight-for-height (%)	77.1 (12.0)	78.3 (12.0)	67.7 (7.8)
Height-for-age (%)	97.2 (7.3)	97.5 (7.2)	94.8 (7.9)

weight-for-height (%) and height-for-age (%) of all 100 children who took part in the study as well as the 89 and 11 children who completed and did not complete the study, respectively. The degrees of wasting and stunting of the 89 children who completed the study are presented in Table 2. Overall, 77 (86.5 %) of the children were wasted and 35 (39.3 %) were stunted with 14 (15.7 %) children both moderately/severely wasted and stunted.

The mean urinary flow rate for the 89 children who provided urine samples was 24.4 mL/h with a range from 8.6 to 45.1 mL/h. Therefore, no child who completed the study was excluded from data analysis.

Tables 3 and 4 show mean (SE) intake, excretion and retention of fluoride and fractional urinary fluoride excretion in the children who provided complete urine samples, stratified by degrees of wasting and stunting, respectively. Generally, TDFI and DUFE were statistically significantly higher in the 77 pooled wasted children (57.7 and 29.7 μg/kgbw/d, respectively) than the 12 non-wasted children (39.4 and 17.0 μg/kgbw/d, respectively) (Table 3). The mean TDFI was statistically significantly higher in the severely wasted (72.2 μg/kgbw/d) than the mildly- (43.3 μg/kgbw/d) and moderately-wasted (54.4 μg/kgbw/d) children. The mean DUFE was also statistically significantly higher in the severely wasted (37.7 μg/kgbw/d) than the mildly- (21.5 μg/kgbw/d), and moderately-wasted (28.0 μg/kgbw/d) children.

As presented in Table 4, TDFI and DUFE were statistically significantly higher in the stunted (65.1 and 34.5 μg/kgbw/d, respectively) than the non-stunted children (48.8 and 23.7 μg/kgbw/d, respectively). Nevertheless, the mean TDFI was statistically significantly lower in the mildly-stunted (59.8 μg/kgbw/d) than the moderately-stunted children (77.7 μg/kgbw/d).

The mean FUFE was similar in the wasted and non-wasted children (Table 3) as well as in the stunted and non-stunted children (Table 4), with no statistically significant differences in FUFE among different categories of wasting or stunting.

Table 5 shows the mean (SD) fluoride concentration of the childrens' nails, stratified by degrees of wasting and stunting. No statistically significant differences in fluoride concentration of either fingernail or toenail were found among non-wasted and pooled wasted children, although the mean fingernail fluoride concentration was statistically significantly higher in severely-wasted (5.0 μg/g) than non-wasted children (3.1 μg/g). The mean fingernail fluoride concentration was statistically significantly higher in pooled stunted children (5.4 μg/g) than non-stunted children (3.5 μg/g), whereas there were no statistically significantly differences in toe-nail fluoride concentration between the two groups.

Table 2
Distribution of stunting and wasting in the children who completed the study (n = 89).

Nutritional status		Stunting				
		Normal	Mild	Moderate	Severe	All
Wasting	Normal	12 (13.5)	0	0	0	12 (13.5)
	Mild	13 (14.6)	3 (3.4)	0	0	16 (18.0)
	Moderate	26 (29.2)	9 (10.1)	0	2 (2.2)	37 (41.6)
	Severe	3 (3.4)	9 (10.1)	12 (13.5)	0	24 (26.9)
	All	54 (60.7)	21 (23.6)	12 (13.5)	2 (2.2)	89 (100)

Table 3
Mean (SE) intake, excretion and retention of F and fractional urinary F excretion, stratified by degrees of wasting, in children providing complete samples (n = 89).

Parameters	Non-wasted (n = 12)	Degree of wasting			
		Mild (n = 16)	Moderate (n = 37)	Severe (n = 24)	Pooled* (n = 77)
Total daily F intake (TDFI; μg/kgbw/d)	39.4 (6.8) [a, A]	43.3 (5.6) [b]	54.4 (3.7) [c]	72.2 (4.6) [a,b,c]	57.7 (2.8) [A]
Daily urinary F excretion (DUFE; μg/kgbw/d)	17.0 (5.5) [a, A]	21.5 (4.5) [b]	28.0 (3.0) [c]	37.7 (3.7) [c a, b,c]	29.7 (2.1) [A]
Fractional urinary F excretion (FUFE; %)	47 (5)	48 (4)	50 (3)	49 (4)	49 (18)

Mean (95 % confidence intervals) differences and statistically significant P-values using a general linear model (GLM) which included a covariate of altitude:
TDFI: (a) severely- vs non-wasted; 32.8 (16.2, 49.3); P < 0.001; (b) severely- vs mildly-wasted; 28.8 (14.4, 43.2); P < 0.001 and (c) severely- vs moderately-wasted; 17.8 (6.1, 29.5); P = 0.003; and (A) non-wasted vs pooled-wasted; 18.8 (2.8, 34.8); P = 0.022.
DUFE: (a) severely- vs non-wasted; 20.7 (7.3, 34.0); P = 0.003; (b) severely- vs mildly-wasted; 16.1 (4.5, 27.7); P = 0.007 and (c) severely- vs moderately-wasted; 9.7 (0.2, 19.); P = 0.044; and (A) non-wasted vs pooled-wasted; 12.9 (0.7, 25.3); P = 0.039.
* Mild + Moderate + Severe.

Table 4
Mean (SE) intake, excretion and retention of F and fractional urinary F excretion, stratified by degrees of stunting, in children providing complete samples (n = 89).

Parameters	Non-stunted (n = 54)	Degree of stunting			
		Mild (n = 21)	Moderate (n = 12)	Severe (n = 2)	Pooled* (n = 35)
Total daily F intake (μg/kgbw/d)	48.8 (3.2) [a, A]	59.8 (5.0) [b]	77.7 (6.7) [a,b]	43.1 (16.5)	65.1 (4.1) [A]
Daily urinary F excretion (μg/kgbw/d)	23.7 (2.5) [a, A]	30.6 (4.0)	43.0 (5.3) [a]	25.2 (13.0)	34.5 (3.1) [A]
Fractional urinary F excretion (%)	48 (2)	51 (4)	49 (5)	56 (12)	51 (3)

Mean (95 % confidence intervals) differences and statistically significant P-values using general linear model (GLM) which included a covariate of altitude:
TDFI: (a) moderately- vs non-stunted; 28.9 (14.0, 43.8); P < 0.001; (b) moderately- vs mildly-stunted; 17.9 (1.2, 34.5); P = 0.036; and (A) non-stunted vs pooled-stunted; 16.3 (5.8, 26.8); P = 0.036.
DUFE: (a) non-stunted vs moderately-stunted; 19.2 (7.5, 30.9); P = 0.002; and (A) non-stunted vs pooled-stunted; 10.8 (2.7, 18.9); P = 0.01.
* Mild + Moderate + Severe.

4. Discussion

To the best of our knowledge this is the first study to compare FUFE in young children with different degrees of wasting and stunting.

The overall mean weight (15.7 kg) and height (100.5 cm) of children, in our study, was slightly lower than the corresponding values of 17.1 kg and 105.8 cm reported for a universal similar age group according to WHO child-growth standards [20]. We were unable to identify such data for a similar age group, in Nepal, with which to compare our findings.

Table 5
Mean (SE) F concentration of nails, stratified by degrees of wasting and stunting, in children proving nail samples [n = number of children providing the sample].

F concentration (μg/g):	Non-affected	Degree of wasting or stunting			
		Mild	Moderate	Severe	Pooled*
Wasting					
Fingernail F concentration (μg/g)	3.1 (0.8) [i] [n = 7]	4.0 (0.7) [n = 8]	4.0 (0.4) [n = 21]	5.0 (0.5) [i] [n = 15]	4.4 (0.3) [n = 44]
Toenail F concentration (μg/g)	2.7 (0.7) [n = 8]	4.5 (0.8) [n = 7]	4.4 (0.5) [n = 20]	3.6 (0.5) [n = 13]	4.2 (0.3) [n = 40]
Stunting					
Fingernail F concentration (μg/g)	3.5 (0.3) [a,] [n = 31]	6.0 (0.5) [a,b] [n = 13]	4.1 (0.7) [b] [n = 7]	– [n = 0]	5.4 (0.4) [A] [n = 20]
Toenail F concentration (μg/g)	4.2 (0.4) [n = 32]	3.5 (0.7) [n = 9]	2.6 (0.8) [n = 6]	6.1 [n = 1]	3.3 (0.5) [n = 16]

Mean (95 % confidence intervals) differences and statistically significant P-values using general linear model (GLM) which included a covariate of altitude: (i) non-wasted vs severely-wasted; 2.1 (0.3, 4.1); P = 0.026.
(a) non-stunted vs mildly-stunted; 2.5 (1.2, 3.7); P < 0.001; (b) mildly-stunted vs moderately-stunted; 2.0 (0.2, 3.7); P = 0.029; and (A) non-stunted vs pooled stunted; 1.8 (0.7, 3.0); P = 0.002.
* Mild + Moderate + Severe.

However, a study in six-year-old Nepalese children living in the Kathmandu valley reported a mean body mass of 17 kg and a height of 107.3 cm [21]; slightly higher than the corresponding values for the 4−5 year olds in our study. These differences could be due to the difference in age between the groups of children studied.

In our study, 42 % of the children were stunted, compared with a slightly lower prevalence of 36 % reported by The World Bank for Nepalese children <5 years old in 2016 [14]. Our study also found that 17 % of the children were moderately- to severely-stunted in comparison with the corresponding figure of 12.5 % reported for 4−5-year-olds in Kathmandu [22].

However, the prevalence of moderate- to severe- wasting in our studied children (71 %) was considerably higher than the corresponding figures of 9.6 % reported by the World Bank for <5 year old Nepalese children [14] and 14.2 % reported for 4−5 year old children in Kathmandu [22]. Our results suggest that acute undernutrition could be a critical public health concern in children aged 4−5 years, living in Banepa and Rajbiraj.

The overall mean TDFI was significantly higher in wasted (Table 3) and stunted (Table 4) children compared with non-affected children. However, the mean TDFI for all groups of children, in our study, was lower than the upper tolerable intake level (UL) of 0.1 mg F/kg bw/d, a suggested value based on a prevalence of less than 5% of moderate dental fluorosis in children. Water has been reported as the primary source of fluoride exposure in some communities including Nepal [15]. Therefore, the higher TDFI in wasted- and stunted-children, found in our study, could be due to a higher than average consumption of water.

Our study also found a significantly higher DUFE in wasted and stunted children compared with non-affected children, which could be explained by higher total fluoride intake in these children. Similarly, a statistically significantly (P = 0.003) greater urinary fluoride concentration has been reported in underweight participants aged 11–20 years in a cross-sectional study in Mexico [11]. The Mexican study also reported that 42 % of those with severe dental fluorosis (TFI ≥ 6) were underweight, compared with 19 % of those having TFI < 6 [11]. However, the latter study did not measure total fluoride intake of the study participants, an important parameter influencing urinary excretion of fluoride as well as severity of dental fluorosis.

It is estimated that almost 45 % of the daily fluoride absorbed by healthy children is excreted in the urine which is the main route for fluoride removal from the body [6]. Our study showed that 47%–52% of ingested fluoride was excreted in the urine with no statistically significant difference in FUFE between non-affected and stunted/wasted children. These findings indicate that fluoride absorption and urinary excretion were not affected by nutritional status in this group of children.

Since fluoride enters the nail primarily through its growth end, the concentration of fluoride in nails reflects the average level of fluoride intake and plasma concentration over a prolonged period [23]. Our study showed no statistically significant differences in fluoride concentration of toenail among different groups (non-affected, stunted and wasted); whereas the mean fingernail fluoride concentration was statistically significantly higher in stunted children than non-stunted children. Our findings also indicated a significantly higher concentration of fluoride in fingernails of severely-wasted children compared with non-wasted children. However, these findings should be interpreted cautiously due to a relatively small number of non-wasted children (who provided nails samples) in this study. Although both finger- and toe-nails have been suggested as potential biomarkers for monitoring acute, sub-chronic and chronic exposure to fluoride, some reports have concluded fingernail to be a less reliable biomarker of fluoride body burden, being prone to external contaminations [23,24].

Our study is the first to compare total daily intake and urinary excretion of fluoride as well as fluoride concentrations in nail clippings in wasted and stunted children. The main strengths of our study are the detailed assessment of total fluoride intake from all sources and thorough measurement of urinary fluoride excretion over a 24 h period. However, the present study has several limitations which could be addressed by future studies. The first limitation is the sample size; in particular the comparatively small number of children who were both stunted and wasted either moderately or severely (n = 14). The present study concentrated on a narrow age group (4−5 year-olds). Since fluoride metabolism and nutritional status can be affected by age, more studies are needed to investigate the effect of nutritional status in other age groups of children, particularly younger children. Additionally, in the present study, we did not conduct any clinical dental examinations. Although currently a consensus exists for a positive association between malnutrition and prevalence of dental fluorosis, this association should be investigated further due to the limitations of previous studies and their contradicting findings. For example, a study in Saudi Arabia reported a relationship between nutritional status (calculated from height-for-age) and prevalence of enamel lesions in 2- to 6-year-old Saudi boys [8] whereas a study in Brazil reported that dental fluorosis was independent of nutritional status when assessed by height-for-age and weight-for-age [25]. None of the previous studies measured nutritional status of children during the period of maximum susceptibility to fluorosis in the maxillary central incisors, i.e. the first three years of life [1].

In conclusion, our study found a significantly higher fluoride intake, and consequently higher urinary fluoride excretion, in wasted and stunted children. However, the proportion of ingested fluoride excreted in urine (and consequently the proportion retained in the body) was not affected by nutritional status. These findings suggest that nutritional status may be less likely to be a main risk factor for the development of dental fluorosis than children's dietary habits or total fluoride intake. However, to fully understand the effect of nutritional status on dental fluorosis, longitudinal studies are needed to assess nutritional status as well as other factors which could affect dental fluorosis during the tooth formation period.

Authors' contributions

FVZ, AM, and OS conceived and designed the study; OS collected and analysed the samples; FVZ supervised the project with help from AM;

FVZ and OS analysed the data and AM contributed to the interpretation of the results; FVZ took the lead in writing the manuscript. All authors read, provided critical feedback and approved the submitted paper.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

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