Factors associated with dental fluorosis amongst Malaysian children exposed to different fluoride concentrations in the public water supply

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Abstract

Objectives: To determine the prevalence of dental fluorosis, and factors associated with its occurrence in two cohorts of children exposed to different fluoride concentrations in the Malaysian water supply.

Methods: A cross sectional study was conducted among lifelong residents (n = 1155) aged 9 and 12 years old living in fluoridated and non-fluoridated areas. Malaysian children aged 12 years were born when the level of fluoride in the public water supply was 0.7ppm whilst those aged 9 years were born after the level was reduced to 0.5ppm. Fluorosis was blind scored using standardized photographs of maxillary central incisors using Dean's criteria. Fluoride exposures and other factors were assessed by parental questionnaire. Data were analyzed using descriptive statistics, Chi-squared analyses and logistic regression.

Results: Fluorosis prevalence was lower (31.9%) among the younger children born after the reduction of fluoride concentration in the water, compared to a prevalence of 38.4% in the older cohort. Early tooth brushing practices and fluoridated toothpaste were not statistically associated with fluorosis status. However, the prevalence of fluorosis was significantly associated with parents' education level, parents' income, fluoridated water, type of infant feeding method, age breast feeding ceased, use of formula milk, duration of formula milk intake, and type of water used to reconstitute formula milk via simple logistic regression. Fluoridated water remained a significant risk factor for fluorosis in multiple logistic regression.

Conclusion: Fluorosis was lower among children born after the adjustment of fluoride concentration in the water. Fluoridated water remained as a strong risk factor for fluorosis after downward adjustment of its fluoride concentration.

Key words: dietary fluoride, dental fluorosis, risk factors, water fluoridation

Introduction

The anti-caries effect of fluoride has been well established and has contributed to the decline in caries prevalence worldwide. At low concentrations in drinking-water, fluoride has beneficial effects on teeth, although excessive exposure can increase the risk of dental fluorosis. Balancing the benefits and risks of fluoride is complex because the fluoride intake important to caries prevention generally occurs after tooth eruption, whereas fluoride intake important to the increased risk of dental fluorosis occurs before the eruption period (1). Fluorosis is an indicator of excess fluoride intake during the critical period of tooth development in early childhood (2,3). During tooth development, unerupted permanent teeth are more sensitive to fluorosis development between the first 2 to 3 years of life for maxillary central incisors and the first 6 to 8 years of life for posterior teeth (2). During this period, the dietary sources of fluoride are from reconstituted infant formula, weaning food and water, while the main non-dietary source of fluoride is from toothpaste.

Studies have reported that early use of toothpaste, higher brushing frequency (more than once per day) (4), a larger quantity of toothpaste dispensed, swallowing toothpaste in infancy and higher fluoride toothpaste concentration have all been reported as risk factors for fluorosis (3). However, a Cochrane review could not confirm an association between the use of fluoride toothpaste and the prevalence of fluorosis (5). Amongst the risk factors, drinking water is considered as the most important exposure of fluoride as it can be consumed either by itself or in beverages and foods (6). For instance, water is also consumed when preparing powder based infant formula and weaning food. Experimental studies reported that fluoride content in breast milk (7,8) and infant formula is low (9-12). However, some data have also shown fluoride content of formula milks to be quite high, especially soy-based products (10, 11,13). With regard to reconstituted infant formula, the type of water (i.e. its fluoride concentration) used in preparation of infant formula and weaning food is reported to be associated with fluorosis (11,14,15). Several studies reported that infants fed with breast milk are less likely to develop dental fluorosis (16,17). Nevertheless, an epidemiological study in Australia (18) reported contradictory findings in which infant formula use in non-fluoridated areas was associated with a high prevalence of fluorosis, whereas in fluoridated areas this was not the case. Indeed, this association persisted after controlling for other fluoride sources (18). These mixed findings reflect the different factors that can affect an individual's susceptibility to fluorosis and they show an ongoing need for updates on the evidence of the balance of risks and benefits of fluoride use.

As drinking water is one of the main exposures to fluoride, any changes in its fluoride concentration may alter total daily fluoride intake for an individual. The World Health Organization guideline has set the fluoride concentration at a recommended range of 0.5 to 1.5 ppm for artificial fluoridation (19). It emphasized that this value set in the guidelines is intended to be adapted to take account of local conditions in specific countries. In past years, some countries such as Hong Kong (20), the United States of America (21) and Ireland (22) have adjusted the fluoride concentration in the water downwards. Similarly, there was a change in the public health policy in Malaysia with regard to concentration of fluoride in the water, i.e., when in 2005 the concentration was reduced from 0.7ppm to 0.5ppm (23). This situation warranted further investigation to determine whether the prevalence of fluorosis and factors affecting the occurrence of fluorosis changed following this downwards adjustment.

Dental fluorosis and its associated risk factors have been described previously in the literature (3, 15, 17). More recent evidence on fluorosis mostly focuses on naturally fluoridated water which is beyond the upper limit of 1.5ppm (24). However, there is limited evidence relating the effect of lowering fluoride level in the water supply on dental fluorosis. A series of surveys in Hong Kong reported that a decline in the concentration of fluoride in the drinking water (1.0ppm in 1970, 0.7ppm in 1978 and 0.5ppm in 1988) led to reductions in fluorosis prevalence (20). However, they also report that the prevalence of fluorosis then went on to increase in 2010 despite fluoride levels remaining constant at 0.5ppm (20). This study did not report any factors associated with fluorosis other than changes to fluoride concentrations in the water.

Thus, the present study aims to determine the factors associated with fluorosis occurrence, in two cohorts of children exposed to different fluoride concentrations in the Malaysian water supply.

Methods

Ethical considerations

The present study was part of a larger study which evaluated the impact on oral health of this reduction of fluoride concentration in the water supply in Malaysia. The protocol for the study was approved by the Research Ethics Committee, School of Dentistry, Cardiff University [DSREC 14/17a] and permission to conduct the study was obtained from relevant authorities in Malaysia namely, the Ministry of Health, Ministry of Education and State Education Department. The data were collected from January 2015 to May 2015.

Study population

This study was cross sectional in design and it involved primary school children aged 9 and 12 years. The downward adjustment of fluoride concentration from 0.7 to 0.5 ppm occurred in early December 2005. All children were lifelong residents enrolled in public schools in the states of Negeri Sembilan (fluoridated at 0.7 then 0.5ppm) and Kelantan (non-fluoridated, 0 ppm). Two birth cohorts of 9 and 12 years of age included in this study were children born between January 1, 2006 to December 31, 2006 (after the reduction in fluoride levels) and children born between January 1, 2003 to December 31, 2003 (before the reduction in fluoride levels). The 12-year-old group in fluoridated area were exposed to 0.7ppm in the first 23 to 35 months of their life. This exposure was calculated depending on their date of birth (Figure 1). The age groups (9 and 12 years) were based on school term in Malaysia which commenced in January. The minimum sample size calculated with a 90% power, a statistical significance level of 0.05, a confidence interval level of 95%, and prevalence of mild fluorosis at 17.8% within the population was 227 for each age group per area. The sample size was inflated to 400 per subgroup. In total, 1600 children from 16 public schools were invited to participate in this study. Details of the sampling method and sample size calculation have been reported elsewhere (25).

Concentrations of fluoride level in the water

Concentrations of fluoride in the water was taken from the state and national water quality reports which confirmed that the fluoride levels in Negeri Sembilan consistently complies with the national standard of 0.5 ppm 0.5 ppm ± 0.2 ppm. This, justifies choosing this state as a fluoridated community. According to national data only 25% of districts in the Kelantan area were fluoridated (26) and these were excluded from the sampling frame to serve as the non-fluoridated area (0 ppm).

Data collection

Parents who gave signed informed consent for their children to participate were asked to complete a questionnaire. This consisted of questions about the child's infant feeding patterns, oral hygiene practices at age less than six years and demographic background. Questions on infant feeding practices were divided into breast-feeding and formula-feeding practices. If the child was breast-fed, this related only to the age at which breast feeding finished. If the child was ever fed infant formula, this related to the age the child started and finished formula feeding and the type of water used to prepare the infant formula (i.e. tap, filtered, bottled). Filtered tap water in this context referred to a range of different types of water filtration system (with or without the ability to remove fluoride). Questions about oral hygiene practices included: the age at which the child started toothbrushing with toothpaste, toothbrushing supervision, frequency of brushing, behavior after brushing, habits of eating and licking toothpaste, amount and the type of toothpaste used.

Clinical examination of fluorosis was conducted on maxillary central incisors using Dean's Index by a trained and calibrated examiner. Digital images of the maxillary incisors were taken to enable blind scoring of dental fluorosis. Anterior teeth were examined and photographed in a wet condition. Children were asked to moisten their teeth or if it was not possible damp cotton wool was used to keep the teeth moist. Standardized digital images of maxillary central incisors were taken using a digital SLR camera, Nikon D3300 body, Sigma 105mm f/2.8 macro lens, Sigma ring flash EM 140DG. Most of the digital photographs only involved one exposure per child. However, additional exposures were attempted when the first image was not satisfactory. On occasions where more than one exposure had been taken, the best quality image was selected for scoring. The images were mixed randomly using a unique identifier for blind scoring. All examiners underwent training on fluorosis scoring using an on-line training module developed by the University College Cork, Ireland (www.fluorosisindex.com). Examiners were blinded from the status of child's area of residence. The fluorosis outcome measure was the consensus score from the digital photographs by three examiners using Dean's Index. A total of 111 images were used in a calibration exercise between the three photographic examiners (NAMN, BLC, IGC). All three examiners scored the photographs at the same time individually, followed by discussion for consensus score for each image. The kappa score for inter-examiner reliability between individual and consensus score for each examiner was substantial ranging from 0.83 to 0.91. The same procedure was used for all images included in the present study. The details about examiner reliability using photographic scoring method have been published earlier (27). The blinded digital photographic scoring of fluorosis aimed to minimize bias from clinical scoring.

Data analysis

The presence of fluorosis was defined by a Dean's score that was greater than or equal to 2, which included the categories of "very mild" or greater. Data were analyzed using descriptive, chi-square analyses, and binary logistic regression. The selection of variables to test for association with fluorosis was based on the exposure to fluoride during the developmental (at risk) stages of the central incisors. Odds Ratios (OR) and 95% confidence intervals were obtained initially by using simple binary logistic regression, i.e., one dependent variable (fluorosis) is regressed upon only one independent variable. In order to adjust for potentially confounding effects, multiple binary logistic regression, i.e., one dependent variable (fluorosis) is regressed upon more than one independent variable. Variables with significant association ($p < 0.05$) at bivariate analysis were further analyzed using multivariate logistic regression to compensate for potentially confounding effects. These variables were entered in one block using the Enter method. Interaction was also tested between inter-dependent factors to test their contribution to a model. If any interaction was contributory, it was retained and reported. All calculations were carried out using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Results

In total, 1155 children were dentally examined clinically, including photographs, and the questionnaire was completed by parents/guardians. Of these, 607 children resided in a fluoridated area, and the remaining 548 were from a non-fluoridated area (Figure 2). However, twelve photographs were excluded due to poor quality, which resulted in 1143 photographs that were suitable for analysis. The prevalence of fluorosis defined by Deans Index ≥ 2 was 35.7% in the fluoridated communities (for both 9- and 12-year-olds), which was significantly higher ($p < 0.001$) than the prevalence of 5.5% for those residents in non-fluoridated communities for both age groups. Of those in the fluoridated community, the prevalence of fluorosis was higher among the older age group (38.4%) than the younger age group (31.9%). Details of the distribution and severity of fluorosis has been reported in an earlier publication (21).

Table 1 shows the results of simple logistic regression analysis of the prevalence of fluorosis as a function of demographic characteristics for all photographs that were eligible for analysis (n = 1143). Children whose parents had been educated only at primary school education (or less) had significantly lower fluorosis prevalence than those whose parents had a college or university education. Girls had more fluorosis than boys, although the difference was not statistically significant. Results of simple logistic regression of fluorosis with respect to feeding practices are presented in Table 2. Children who were fed by infant formula (either fully fed by formula or mixture of breast + formula feed) were more likely to have fluorosis than those who had been breast fed exclusively (no exposure to infant formula). Children who started formula at an earlier age, finished formula at a later age and a had longer duration of formula use were significantly associated with a higher fluorosis prevalence.

Finally, Table 3 shows the results of simple logistic regression analysis of fluorosis with respect to oral hygiene habits at age less than six years among the study participants. There was evidence of some variation in terms of early childhood oral hygiene practices with fluorosis status. For example, fluoridated toothpaste, age started toothbrushing and age started toothbrushing with toothpaste before 2 years old were associated with a higher fluorosis prevalence. However, the associations were not statistically significant. Exposure to fluoride from the water was found to be significantly associated with a high fluorosis prevalence in comparison to those who lived in non-fluoridated areas.

Table 4 shows results of the multiple logistic regression for all photographs that were eligible for analysis (n = 1143). The 12-year-old children who had been exposed to (0.7ppm in the first two years of life then 0.5ppm for the rest of their lives) showed an increased prevalence of fluorosis compared to those with no exposure, i.e., non-fluoridated (OR = 9.12, 95% CI = 5.15 to 16.14). The 9-year-old children exposed to the lower fluoride level (0.5ppm) throughout their lives also experienced an increased prevalence of fluorosis compared to no exposure, i.e., non-fluoridated (OR = 5.97, 95% CI = 3.32 to 10.72). Use of tap water compared to bottled water resulted in increased chances of fluorosis (OR = 9.90, 95% CI = 1.28 to 76.38), as did filtered tap water compared to bottled water (OR = 8.78, 95% CI = 1.11 to 69.71). Other factors were not significantly associated with fluorosis in the multiple logistic regression model, and this loss of significance might be due to the effect of adjusting for the other mutually confounding variables.

Discussion

Results for the decline in fluorosis prevalence in children exposed to the lower fluoride level in the water shown here, support the 2005 policy change to the national fluoridation scheme. Malaysian national surveys reported fluorosis prevalence as 62.3% in 1999 (28) and 82.4% in 2013 (28) among 16-year-old school children exposed to 0.7ppm throughout life prior to downward adjustment of fluoride in the water supply. However, caution should be taken when comparing the data, as the national surveys were reported using full mouth Dean's fluorosis scores. Fluorosis data in the present study on central incisors may underestimate the true fluorosis prevalence in the study population. However, photographic scoring helps to minimize examiner bias and the central incisors were regarded as the teeth most likely to cause aesthetic concern.

Higher fluorosis prevalence in the present study was found to be significantly associated with higher parental income and education level via simple logistic regression. The link between socio-economic status and fluorosis has also been reported by other studies in Brazil (30) Mexico (31) Pakistan (32). Unlike dental caries, the relationship between fluorosis and socio-economic status has not been fully established in the literature. Results across studies have been inconsistent in terms of fluorosis and socio-economic status with some studies reporting an inverse relationship between the two variables. However, the socio-economic status factors were not significant in the multiple logistic regression in the present study and other studies reviewed (30, 32, 33). Several authors have postulated that a high fluorosis prevalence among affluent families might be due to the ability to purchase fluoride toothpaste (30,33). However, fluorosis prevalence in the present study was strongly associated with exposure to fluoride in the water rather than to fluoride toothpaste and oral hygiene practices. Therefore, a potential reason for the association could be due to more children with parents of higher socio-economic status were exposed to fluoridated water than those of lower socio-economic status.

Findings from simple logistic regression also indicate that children who were breast-fed during infancy were significantly less likely to have fluorosis than those who were formula users. This finding is supported by other studies (16,17). It is known that, even if a mother is consuming fluoridated water, human milk maintains very low fluoride concentrations (< 0.5 μM) due to the limited transfer of fluoride from plasma to breast milk (8,14). Furthermore, longer breast-feeding duration also led to less fluorosis in the current study. Children who had been breastfed for a prolonged period (>12 months) were less likely to develop fluorosis. The same findings have been reported in Canada (16) and in Ethiopia (6). In addition, the practice of breast-feeding was found to be linked with family economic status. For example, in this study, those with low income and low education levels were more likely to breastfeed their children and to do so for longer. These findings confirm results from another local study in Malaysia that has reported a higher prevalence of breast-feeding in lower socio-economic status families, and greater use of infant formula amongst the affluent (34). This could be due to the fact the majority of the mothers of lower socio-economic status were not working outside the house being full time housewives, which makes breast-feeding easier than for those who go out to work. This pattern differs from that observed in the UK (34) and USA (36), where breastfeeding is more common in higher socioeconomic groups.

Those who were fed with infant formula were associated with higher fluorosis prevalence. Based on the international literature, infant formula generally had a low fluoride content after the 1970s (14,15). The low concentration of fluoride level in infant formula varied from (0.28 µg/F g of milk powder) in United Kingdom (11) to (0.41 µg/F g) in Japan (10). Nevertheless, it has been reported that a number of soy-based infant formulas tend to have a higher fluoride content than other milk types (13). A systematic review suggests that fluorosis has a weak association with infant formula because of low fluoride concentration in infant formula (15). However, it shows a strong correlation with the type of water used to reconstitute the formula. Findings from the present study are in agreement with previous studies that reported infant formula reconstituted with fluoridated tap water increases the risk for dental fluorosis (9-11). No information was collected about the type of water filters used among the studied population. It is likely they were not of reverse osmosis type so it can be assumed that they did not have a significant effect on fluoride removal as reported by a previous local study (37). Existing local data reported that fluoride concentration in bottled water ranged from 0.10 to 0.43mg/L (38). It is therefore likely that fluoride concentration of bottled water consumed by the population was relatively low.

In terms of oral hygiene practices, fluoridated toothpaste, age at which children started toothbrushing and the age they started using toothpaste before 2 years old were only associated with higher fluorosis prevalence in the simple logistic regression analysis, the findings may reflect on the lack of accuracy of historical data. A common limitation with this type of study is that it relied on parents' self-reported behavior and recall bias is always a possibility in questionnaire data. Inability to collect accurate historical fluoride intake data is acknowledged as a limitation in this present study. For example, the ability of parents to recall the nature of oral hygiene habits and infant feeding practices were likely to become less accurate with the passage of time. Additionally, although the examiners were blinded towards residential status, those from the younger age groups may be identifiable based on the stage of dental development, apparent on the photographs. Another limitation is that the reporting of concentrations of fluoride in the water relied on the state and national water quality report. Although no attempt was made to validate the fluoride concentration in the water supply, data from the state and national report was considered reliable as it involves a rigorous monitoring process. Hence, findings from the present study should be interpreted with these limitations in mind. Nevertheless, retrospective methods of data collection are commonly reported in the literature because of its practicality, time saving and cost- effectiveness.

The strengths of the present study include a good response rate, sound sampling technique, blind-scoring of fluorosis outcome measure and the confounding factors were controlled in the multiple logistic regression. This study adds knowledge in relation to reduction of fluoride concentration from 0.7 to 0.5ppm on fluorosis outcome in tropical climate country that has wide spread use of fluoridated toothpaste. The findings could be relevant to other tropical and subtropical countries when reviewing their fluoride policy. Future research could address some of the study limitations such as to use longitudinal study design, incorporate measurement of tap water consumption, type of water filters and inclusion of other variables on feeding and dietary habits during early childhood. The present study only focused on children. Future work may consider evaluating the impact of water fluoridation at 0. 5ppm and fluorosis on adults population.

Conclusion

The prevalence of fluorosis was higher amongst children in the fluoridated areas. The prevalence of fluorosis was significantly associated with parents' education level, parents' income, area of residence, type of infant feeding method, age breast feeding ceased, use of formula milk, duration of formula milk intake, and type of water used to reconstitute formula milk in simple logistic regression. However, only exposure to fluoridated water remained a significant risk factor for fluorosis in multiple logistic regression. The change in Malaysian water fluoridation policy from 0.7 to 0.5 ppm has resulted in a decrease in fluorosis prevalence. Continuous monitoring of water fluoridation policy is needed and long-term evaluation is recommended.

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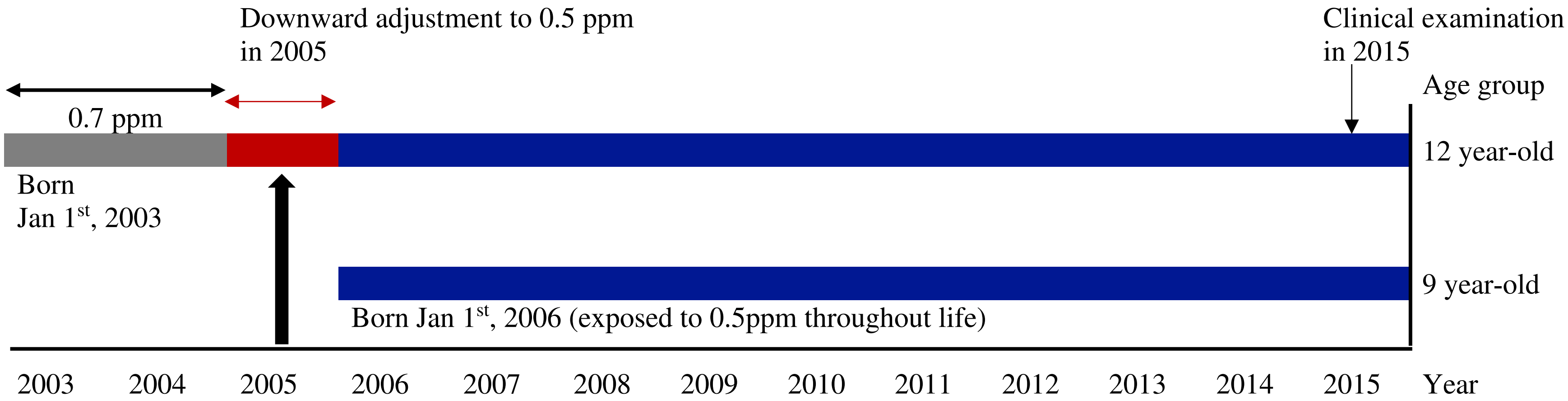
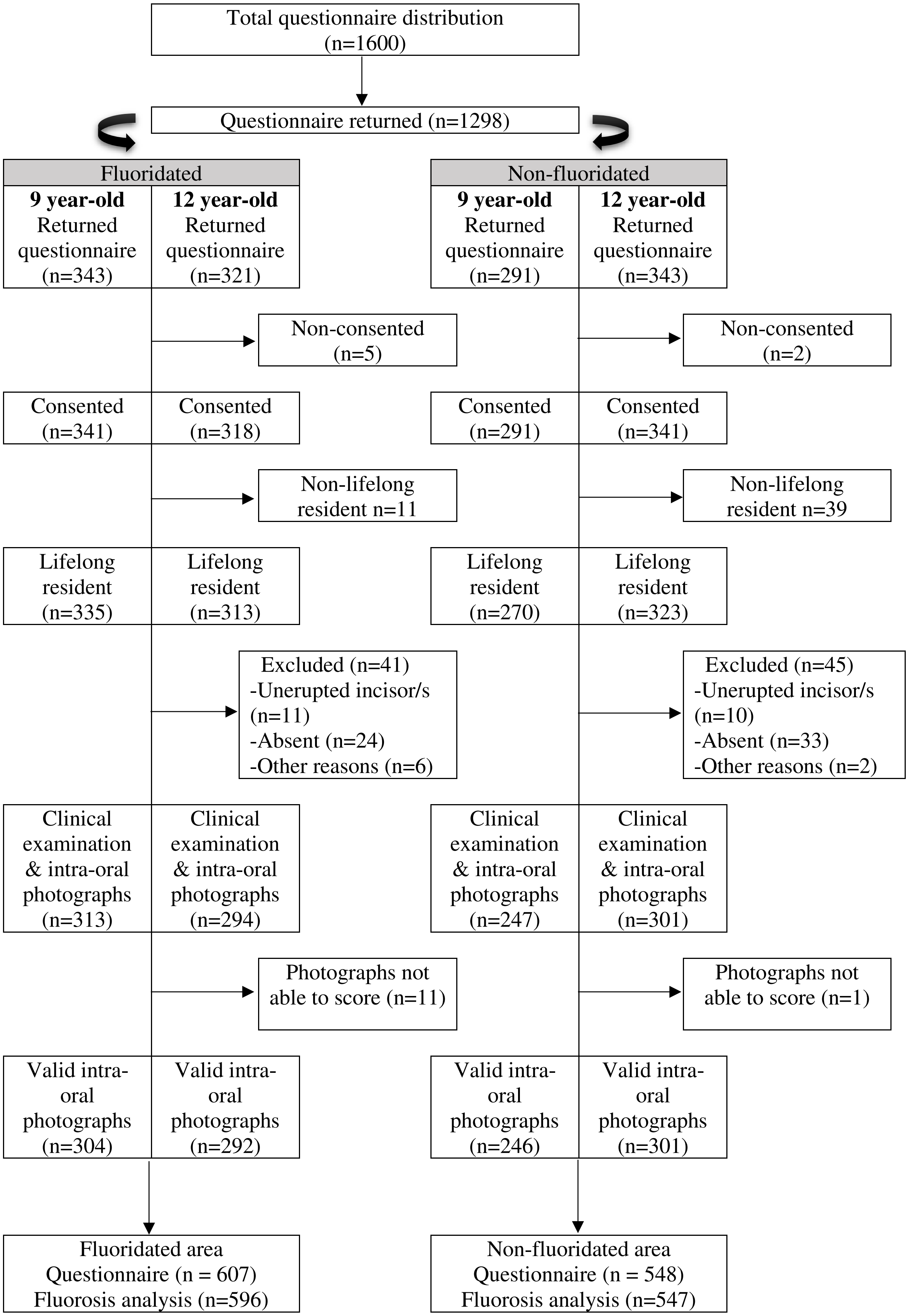
Figure 1 Fluoride exposure from the water among children lived in fluoridated area



Note: Of those living in the fluoridated area, 9 year-olds were exposed to fluoride in the water 0.5ppm throughout lifetime while the 12 year-olds were initially exposed to 0.7ppm and then 0.5ppm. Children in the non-fluoridated had zero exposure to water fluoridation throughout their life.

Figure 2 Flowchart of participants eligibility



TABLES

Table 1 Simple logistic regression analysis of fluorosis prevalence with respect to demographic characteristics of study participants (n = 1143)

Variables (Demographics)	Fluorosis (Deans ≥ 2) *n (%)* Yes	No	OR (95% CI)	P-value
Gender (n=1143)				
Boys	98 (20.0)	393 (80.0)	Reference	
Girls	145 (22.2)	507 (77.8)	1.15 (0.86-1.53)	0.351
Father Education (n=1049)				
College/University	60 (24.0)	190 (76.0)	Reference	
High school	151 (21.7)	546 (78.3)	0.88 (0.62-1.23)	0.447
≤Primary school	12 (11.8)	90 (88.2)	0.42 (0.22-0.82)	**0.011***
Mother Education (n=1080)				
College/University	68 (23.4)	223 (76.6)	Reference	
High school	145 (21.3)	535 (78.7)	0.49 (0.64-1.23)	0.481
≤Primary school	14 (12.8)	95 (87.2)	0.48 (0.26-0.90)	**0.022***
Father monthly income (n=1050)				
≥ MYR 4000	88 (27.2)	235 (72.8)	Reference	
MYR 1000-3999	131 (18.7)	569 (81.3)	0.62 (0.45-0.84)	**0.002***
<MYR 1000	2 (7.4)	25 (92.6)	0.21 (0.05-0.92)	**0.038***
Mother monthly income (n=1090)				
≥ MYR 4000	61 (29.0)	149 (71.0)	Reference	
MYR 1000-3999	60 (22.1)	212 (77.9)	0.69 (0.46-1.05)	0.080
<MYR 1000	104 (17.1)	504 (82.9)	0.50 (0.35-0.73)	**0.000***

*p<0.05, statistically significant

Table 2 Simple logistic regression analysis of fluorosis prevalence with respect to infant feeding practices of study participants (n = 1143)

Variables (Infant feeding practices)	Fluorosis (Deans ≥ 2) n (%) Yes	No	OR (95% CI)	P-value
Use of infant formula (n=1140)				
No	32 (10.4)	277 (89.6)	Reference	
Yes	210 (25.3)	621 (74.7)	2.93 (1.97-4.36)	**< 0.001***
Breast feeding (n=1143)				
No	8 (29.6)	19 (70.4)	Reference	
Yes	235 (21.1)	881 (78.9)	0.63 (0.27-1.47)	0.286
Age breast feeding ceased (n=1119)				
>12 months old	104 (15.3)	574 (84.7)	Reference	
≤12 months old	132 (29.9)	309 (70.1)	2.36 (1.76-3.16)	**< 0.001***
Age started formula (n=841)				
>12 months old	70 (20.8)	267 (79.2)	Reference	
≤12 months old	145 (28.8)	359 (71.2)	1.54 (1.11-2.14)	**0.009***
Age finished formula (n=836)				
>48 months old	125 (29.2)	303 (70.8)	Reference	
≤48 months old	89 (21.8)	319 (78.2)	0.68 (0.49-0.93)	**0.014***
Type of water used to prepare formula (n=830)				
Bottled water	3 (9.4)	29 (90.6)	Reference	
Tap water	162 (25.7)	469 (74.3)	3.34 (1.0-11.11)	**0.049***
Filtered tap water	47 (28.1)	120 (71.9)	3.79 (1.1-13.03)	**0.035***
Duration of formula use (n=827)				
>48 months	85 (32.8)	174 (67.2)	Reference	
≤48 months	125 (22.0)	443 (78.0)	0.58 (0.42-0.80)	**0.001***
Feeding method (n=1140)				
Exclusively formula fed	8 (29.6)	19 (70.4)	Reference	
Combine breast & formula fed	202 (25.1)	602 (74.9)	0.80 (0.34-1.85)	0.597
Exclusively breast fed	32 (10.4)	277 (89.6)	0.27 (0.11-0.68)	**0.005***

*p<0.05, statistically significant

Table 3 Simple logistic regression analysis of fluorosis prevalence with respect to fluoride exposure from water, varnish and oral hygiene habits (established at age less than six years; n = 1143)

Variables (Fluoride exposure)	Fluorosis (Deans≥2) n (%)		OR (95% CI)	P-value
	Yes	No		
Frequency of toothbrushing (n=1137)				
Twice/day or more	138 (21.1)	516 (78.9)	Reference	
Once /day or less	104 (21.5)	379 (78.5)	1.03 (0.77-1.37)	0.861
Supervised toothbrushing (n=1095)				
Never	4 (20.0)	16 (80.0)	Reference	
Yes	234 (21.8)	841 (78.2)	1.11 (0.37-3.36)	0.849
Habits after toothbrushing (n=1126)				
Spat	227 (21.5)	831 (78.5)	Reference	
Swallowed	13 (19.1)	55 (80.9)	0.87 (0.47-1.61)	0.648
Eating/ licking toothpaste (n=1134)				
Never	110 (22.8)	372 (77.2)	Reference	
Yes	131 (20.1)	521 (79.9)	0.85 (0.64-1.13)	0.267
Amount of toothpaste used (n=1134)				
Medium to large	134 (21.2)	497 (78.8)	Reference	
Small	107 (21.3)	396 (78.7)	1.00 (0.75-1.33)	0.988
Type of toothpaste used (n=1112)				
Non-fluoridated toothpaste	28 (20.1)	111 (79.9)	Reference	
Fluoridated toothpaste	210 (21.6)	763 (78.4)	1.09 (0.70-1.70)	0.700
Age started toothbrushing (n=1142)				
After 2 years	161 (20.7)	618 (79.3)	Reference	
Before 2 years	82 (22.6)	281 (77.4)	1.12 (0.83-1.51)	0.460
Age started toothbrushing with toothpaste (n=1134)				
After 2 years	172 (20.7)	657 (79.3)	Reference	
Before 2 years	68 (22.3)	237 (77.7)	1.10 (0.80-1.51)	0.572
Exposure to fluoride in the water supply (n=1143)				
0 lifetime	30 (12.30)	517 (57.4)	Reference	
0.5ppm lifetime	100 (41.2)	204 (22.7)	8.45 (5.45-13.10)	**0.001***
0.7ppm + 0.5ppm	113 (46.5)	179 (19.9)	10.88 (7.03-16.84)	**0.001***
Fluoride varnish (n=804)				
No	147 (22.6)	503 (77.4)	Reference	
Yes	28 (18.2)	126 (81.8)	0.76 (0.49-1.19)	0.231

*p<0.05, statistically significant

Table 4 Multiple logistic regression analysis of fluorosis prevalence (n = 1143)

Explanatory variables	Adjusted OR (95% CI)	P-value
Father education		
College/University	Reference	
High school	0.85 (0.50-1.43)	0.532
≤Primary school	0.74 (0.27-2.04)	0.565
Mother Education		
College/University	Reference	
High school	1.44 (0.83-2.53)	0.198
≤Primary school	1.09 (0.37-3.19)	0.872
Father income		
≥ RM 4000	Reference	
RM1000-3999	0.93 (0.57-1.51)	0.766
<RM 1000	0.29 (0.06-1.54)	0.147
Mother income		
≥ RM 4000	Reference	
RM1000-3999	0.91 (0.48-1.71)	0.763
<RM 1000	0.84 (0.47-1.51)	0.558
Infant formula		
No	Reference	
Yes	0.68 (0.02-23.14)	0.831
Age breast feeding ceased		
>12 months old	Reference	
≤12 months old	1.40 (0.85-2.32)	0.188
Age started formula		
>12 months old	Reference	
≤12 months old	1.10 (0.63-1.92)	0.726
Age finished formula		
>48 months old	Reference	
≤48 months old	1.00 (0.57-1.75)	0.998
Type of water used to prepare formula		
Bottled water	Reference	
Tap water	9.90 (1.28-76.38)	**0.028***
Filtered tap water	8.78 (1.11-69.71)	**0.040***
Duration of formula use		
>48 months	Reference	
≤48 months	0.98 (0.54-1.78)	0.955
Feeding method		
Exclusively formula fed	Reference	
Combine breast & formula fed	0.26 (0.01-5.32)	0.378
Exclusively breast fed	-	-
Fluoride concentration in the water		
0 lifetime (i.e., non-fluoridated)	Reference	
0.5ppm lifetime	5.97 (3.32-10.72)	**<0.001***
0.7ppm in the first two years of life, then 0.5ppm	9.12 (5.15-16.14)	**<0.001***

*p<0.05, statistically significant