# THE EFFECTS OF TEN YEARS OF DEFLUORIDATION ON URINARY FLUORIDE, DENTAL FLUOROSIS, DEFECT DENTAL FLUOROSIS, AND DENTAL CARIES, IN JIANGSU PROVINCE, PR CHINA

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**ABSTRACT: The aim of this study was to evaluate the effect on urinary fluoride, dental fluorosis, defect dental fluorosis, and dental caries of the defluoridation of drinking water for 10 years in Jiangsu province, PR China. In 2002, before defluoridation, 236 children in Wamiao and 290 in Xinhuai aged 8–14 years were recruited and in 2013, after 10 years of defluoridation, a further 68 children in Wamiao and 65 in Xinhuai, aged 8–10 years, were recruited. The effects of defluoridation were evaluated by examining the correlations between the prevalence of dental fluorosis and dental caries, and the urinary fluoride level. The prevalence of dental fluorosis and defect dental fluorosis in 2002 had a significant positive dose–response correlation with the drinking water fluoride with the coefficient correlations, regression equations, and p values being r=0.999, $y=99.552/(1+40.049\times e^{-3.464x})$, and p=0.017; and r=0.987, y=17.520x – 6.950, and p=0.001, respectively. The prevalence of dental fluorosis and defect dental fluorosis were significantly decreased with the decreased drinking water fluoride in Wamiao in 2013 after defluoridation compared with the results in 2002. The prevalence of dental caries and the DMFT were slightly increased in Wamiao in 2013 compared with 2002 and slightly decreased in Xinhuai. However, the levels of DMFT in both villages in 2013 were at a low (Xinhuai) or very-low or low (Wamiao) level. In 2002, the prevalence of dental caries and the DMFT had a U-shaped dose-response correlation with the drinking water fluoride concentration. This study suggests that defluoridation of drinking water is effective for controlling endemic fluorosis in China and that the role of fluoridation of public water supplies for the of control dental caries needs to be further studied.**

Keywords: Defluoridation; Defluoridation evaluation; Dental caries; Dental fluorosis.

## INTRODUCTION

In China, drinking-water-borne endemic fluorosis has been one of the most widely distributed endemic diseases affecting approximately 92 million persons in 1,181 counties.[1-3] By the end of 2010, 81.89% of the villages affected with this condition had changed their water sources to drinking water with a low level of fluoride.[4] In Jiangsu province, 26 counties had drinking-water-borne endemic fluorosis, and, of the total population of 4.46 million, 2.03 million had dental fluorosis and 0.14 million had skeletal fluorosis. By the end of 2010, 86.86% of the endemic fluorosis villages in Jiangsu Province had changed to using a low fluoride drinking water source, but only 70.26% normally used the defluoridated

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water provided in the defluoridation project.[5] As the overall effects of using the low fluoride water sources provided by the defluoridation project had not been assessed, we selected two villages Wamiao, a village with severe drinking-water-borne endemic fluorosis, and Xinhuai, a village without endemic fluorosis, to evaluate the effects of providing low fluoride water for 10 years.

## MATERIALS AND METHODS

The basic information on Wamiao and Xinhuai villages has been published previously.[6-8] Since 2003 in Wamiao and 2009 in Xinhuai almost all residents have used tap water rather than well water. The household shallow wells have been scarcely used with just a few being used for washing but not drinking.[8] Wamiao was a severe endemic fluorosis village with a drinking water fluoride level of 2.47±0.79 mg/L in the household shallow wells, and a range of 0.57–4.50 mg/L.[6,7] Xinhuai was an non-endemic fluorosis village with a well water fluoride level of 0.38±0.21 mg/L and a range of 0.15–0.77 mg/L.[6,7] As a defluoridation project, water from two deep wells has been used as a tap water source of drinking water in Wamiao village since the beginning of 2003. The surface water in Yaohe river has been used as a tap water source in Xinhuai village since 2009.

The studies were conducted between September and December in both 2002 and 2013. We compared the results for dental fluorosis, dental caries, and urinary fluoride before and after the drinking water sources changed during this 10 years.

The subjects in our 2002 investigation have been reported on previously.[6,7] In our 2013 investigation, 8–10 year-old-children, born after December 2003 who had used the low fluoride tap water source for their drinking water were recruited. Seventy-one children were recruited in Wamiao village and 67 in Xinhuai village. Children who had been absent from either village for one year or longer were excluded. This left 68 (95.77% of the number initially recruited) children in Wamiao village and 65 (97.01% of the number initially recruited) in Xinhuai village.

Before the investigation, written informed consent was obtained from the children's parents. The study was approved by the Jiangsu Provincial Center for Disease Control and Prevention, and the Zhejiang Normal University.

We collected 50 mL water and urine samples using plastic bottles which were cleaned by soaking in 10% nitric acid ($HNO_3$) for 48 hr and then washed three times with tap water and twice with distilled water. In the 2013 investigation, the total number of water samples in Wamiao was 14 (2 water samples from each of 5 water taps and 2 water samples from each of two deep wells), and 7 water samples in Xinhuai (5 tap water samples and 2 from Yaohe river sources). The tap water samples were collected according to the five-point sampling methods. The *urina sanguinis* urine samples (50 mL) were collected in the early morning from all the children in the 2002 and 2013 investigations. The water and urine fluoride levels were measured with a fluoride ion selective electrode (Manufactured by Chang Sha Yi Ming Experimental Instrument Co., Ltd, China) with an LOD of 20 μg/L ± 2%.[9,10]

Two dentists and an expert in endemic fluorosis control and prevention examined the children's permanent teeth for dental fluorosis and dental caries with a mouth mirror, forceps, and a probe under sufficient natural light. Before the examination, alcohol cotton swabs were used to clean the surface of the tooth, and keep the tooth surface drying.

Dean's classification and the Chinese "Clinical diagnostic standard for dental fluorosis" (WS/T208-2001)[11] were used for diagnosing dental fluorosis. Dean's six grade classification scale was published in our previous report.[6] Statistical analysis of the prevalence of dental fluorosis was made according to the rates of dental fluorosis (DF, %) by Dean's classification and the rates of defect dental fluorosis (DDF, %) by the WS/T208-2001 diagnostic standard. Defect means there was a small dent, or/and a large pit, or/and a larger striped area in the surface of the dental enamel. Defect dental fluorosis included some "moderate" dental fluorosis (grade 3) and all "severe" dental fluorosis (grade 4) as diagnosed by Dean's criteria.

$$\text{Prevalence of dental fluorosis (\%)} = \frac{\text{Number of children with very mild dental fluorosis or higher}}{\text{Number of children investigated}} \times 100$$

$$\text{Prevalence of defect dental fluorosis (\%)} = \frac{\text{Number of children with defect dental fluorosis or higher}}{\text{Number of children investigated}} \times 100$$

Dental caries in children were mainly crown surface caries, so in this study we only analysed the prevalence of crown surface dental caries. The dental caries were diagnosed under sufficient natural light according to the diagnostic criteria published in *Cariology* edited by Fan Mingwen and Bian Zhuan.[12] The prevalence of dental caries and the mean caries experience (decayed, missing and filled permanent teeth, DMFT) were calculated by the following equations:

$$\text{Prevalence of dental caries (\%)} = \frac{\text{Number of children with dental caries}}{\text{Number of children investigated}} \times 100$$

$$\text{Mean caries experience (\%)} = \frac{\text{Total number of decayed, missing, and filled teeth}}{\text{Number of children investigated}} \times 100$$

The data were entered into Epidata 3.1 (available from http://www.epidata.dk/download.php, The Epidata Association, Odense, Denmark) twice by two undergraduate students, and then subjected to logic checking with the software. The data were analyzed by SAS software (version 8.2; SAS Institute Inc., Cary, NC, USA). The arithmetic mean (AM) was used to describe the measurement data and the comparison of two groups was made using the Independent-Sample T-test.

Constituent ratio was used to describe the numeration data and the comparison of two groups or more was made using the Chi-Square test. We used the CurveExpert version 1.37 Software (Microsoft Corporation, 1993) to analysis the dose-response relationship.

## RESULTS

*The exposure indicators:* The fluoride levels in the tap (drinking) water and in children's urine in 2013 in the Wamiao and Xinhuai villages after the changing of drinking water sources, in 2003 and 2009 respectively, are shown in Table 1.

**Table 1.** Levels of fluoride (mg/L) in the children's urine and the tap (drinking) water in the Wamiao and Xinhuai villages in the 2013 investigation

| Village | Fluoride level in children's urine (mg/L) | | | Fluoride level in tap water (mg/L) | | |
|---|---|---|---|---|---|---|
| | No. of samples | Mean±SD | Range | No. of samples | Mean±SD | Range |
| Wamiao | 68 | 1.39±0.66* | 0.40–3.19 | 14 | 0.91±0.02† | 0.86–0.95 |
| Xinhuai | 65 | 1.30±0.65 | 0.36–2.85 | 7 | 0.89±0.03 | 0.85–0.95 |

*No significant differences were present between the two villages in the fluoride levels in the children's urine (t=0.87, p=0.386); †No significant differences were present between the two villages in the fluoride levels in the drinking water (t=1.58, p=0.13.

In the 2002 investigation, the drinking water fluoride levels in household shallow wells in Wamiao village were significantly higher than those in Xinhuai village (t=44.97, p<0.001)[7] but in the 2013 investigation no significant difference was found in the fluoride levels in the tap water between these two village (t=1.58, p=0.13, Table 1).

In the 2002 investigation, before the changing of the drinking water sources, the levels of urinary fluoride levels in Wamiao village (3.46±1.74 mg/L) were significant higher than those in Xinhuai village (1.01±0.40 mg/L, t=23.17, p<0.001)[7] but in the 2013 investigation no significant differences were present between the two villages (t=0.87, p=0.386, Table 1).

*The effect indicators:* In the 2002 investigation, before the drinking water source changed, the prevalence of dental fluorosis (%) in Wamiao village was significantly higher than in Xinhuai village.[6] When we combined the 2002 data for the two villages, a significant positive dose-response relationship was present between the levels of fluoride in the household shallow well drinking water and the prevalence of dental fluorosis and defect dental fluorosis (Figures 1 and 2).

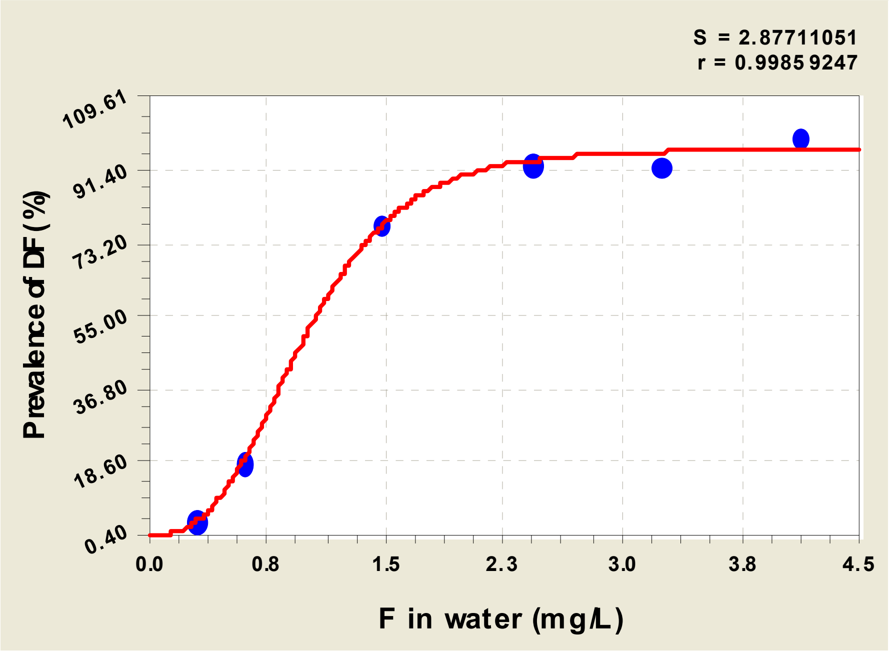


**Figure 1.** The dose-response relationship between relationship between drinking water fluoride (F) in mg/L and the prevalence of dental fluorosis (DF) using the 2002 data for the two villages.

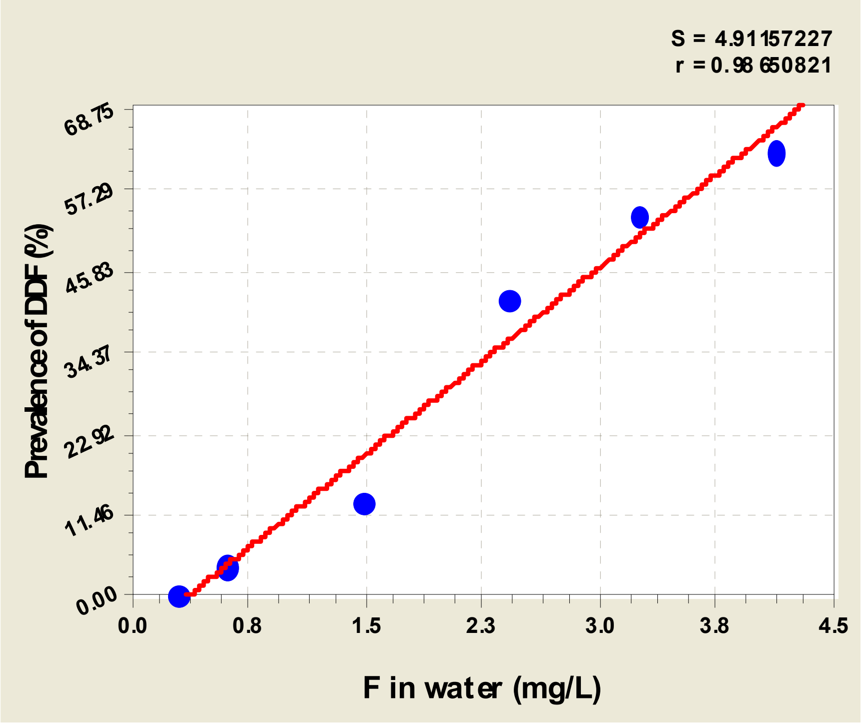


**Figure 2.** The dose-response relationship between drinking water fluoride (F) in mg/L and the prevalence of defect dental fluorosis (DDF) using the 2002 data for the two villages.

Using the logistic model (the optimum selection) to analyse the relationship between the drinking water fluoride and the prevalence of dental fluorosis, we found the coefficient correlation r=0.999 and the regression equation was $y = 99.552/(1+40.049\times e^{-3.464x})$, F=15.580, p=0.017. Using the linear fit model to analyse the relationship between the drinking water fluoride and the prevalence of defect dental fluorosis, we found the coefficient correlation r=0.987 and the regression equation was $y = 17.520x - 6.950$, F=145.245, p<0.001. The prevalence of dental fluorosis was increased quickly as the level of fluoride in the drinking water rose from 0.6 to 2.5 mg/L (Figure 1). When the fluoride level was 2.45 mg/L, the prevalence of dental fluorosis was 93.04%, nearly 100%. As the fluoride level increased further, the prevalence of dental fluorosis showed little change. However, a significant linear dose-response relationship was present between the level of fluoride in the drinking water and the prevalence of defect dental fluorosis (Figure 2).

In our 2013 investigation, no significant difference was found in the prevalence of dental fluorosis between the two villages, Wamiao and Xinhuai (($\chi^2$=3.61, p=0.057, Table 2). After the drinking water sources changed to sources with lower levels of fluoride, the prevalence of dental fluorosis in 2013 was significantly lower than in 2002, especially in Wamiao village.[6] No defect dental fluorosis was found in either of the two villages in the 2013 investigation.

**Table 2.** Prevalence of dental fluorosis (DF) and defect dental fluorosis (DDF) in the 2013 investigation in the Wamiao and Xinhuai villages

| Village | No. of children | No. of children with DF | No. of children with DDF | Prevalence of DF (%) | Prevalence of DFF (%) |
|---|---|---|---|---|---|
| Wamiao | 68 | 6* | 0 | 8.82 | 0.00 |
| Xinhuai | 65 | 2 | 0 | 3.08 | 0.00 |

*No significant difference was present between the two villages in the prevalence of dental fluorosis (DF) ($\chi^2$=3.61, p=0.057);

In our 2002 investigation, the prevalence of dental caries in Xinhuai village was significantly higher than in Wamiao village in males ($\chi^2$=15.74, p<0.001), females ($\chi^2$=4.01, p<0.05), and in the total of males and females ($\chi^2$=18.34, p<0.001, Table 3).[6] According to the WHO epidemic criteria for dental caries, the DMFT was very-low in Wamiao village and low in Xinhuai village.[13]

**Table 3.** The prevalence of dental caries (DC) and decayed, missing, and filled permanent teeth (DMFT) and in the 2002 investigation in Wamiao and Xinhuai villages

| Gender | Village | No. of children | No. of children with DC | Prevalence of DC (%) | DMFT |
|---|---|---|---|---|---|
| Male | Wamiao | 130 | 48 | 36.92 | 0.75 |
| | Xinhuai | 159 | 96 | 60.38* | 1.68 |
| Female | Wamiao | 106 | 42 | 39.62 | 1.09 |
| | Xinhuai | 131 | 69 | 52.67† | 1.63 |
| Total for male and female | Wamiao | 236 | 90 | 38.41 | 0.98 |
| | Xinhuai | 290 | 165 | 56.90* | 1.66 |

*Compared with Wamiao village: *$p<0.001$; †$p<0.05$.

We combined the 2002 data on dental caries from the two villages and divided the children into nine subgroups according to the drinking water fluoride level in their household shallow wells, The dose–response relationships between the drinking water fluoride and the prevalence of dental caries and DMFT are shown in Figures 3 and 4.

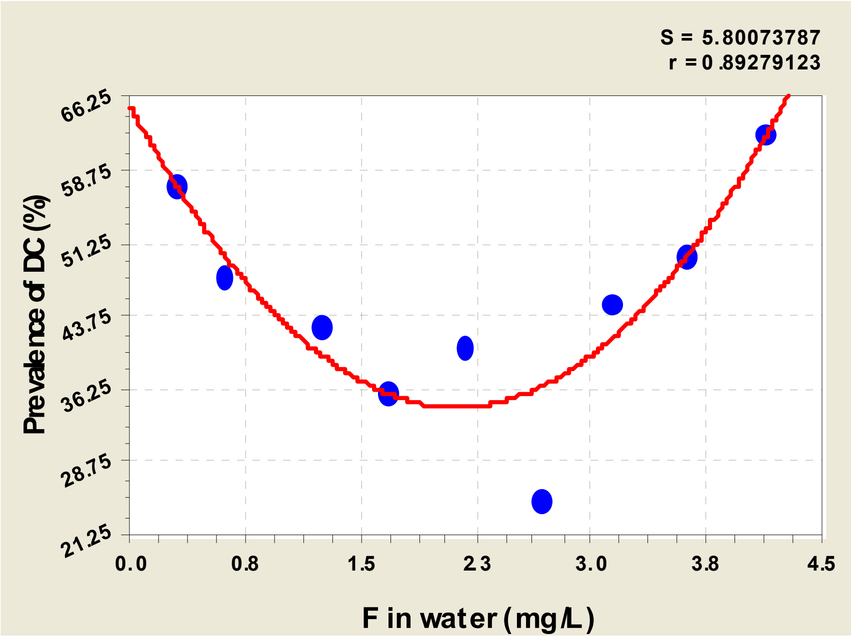


**Figure 3.** The fitted curve of the relationship between water fluoride (F) in mg/L and prevalence of dental caries (DC) by the quadratic model using the 2002 data for the two villages.

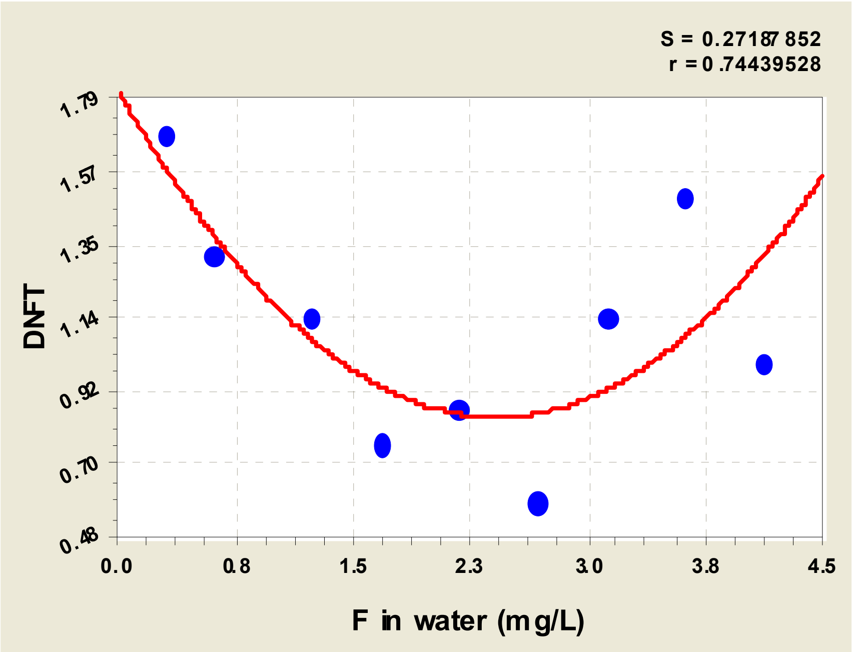


**Figure 4.** The fitted curve of the relationship between water fluoride (F) in mg/L and decayed, missing, and filled permanent teeth (DMFT) by the quadratic model using the 2002 data for the two villages.

The results, using the 2002 data, indicated that when the fluoride concentration in the drinking water was at a relatively low level, the prevalence of dental caries decreased as the fluoride concentration increased. When the fluoride concentration in the drinking water exceeded a certain range, the prevalence of dental caries increased as the fluoride concentration increased further. This possibly desirable range for the fluoride level for minimizing the prevalence of dental cares was found, in this study, to be approximately 1.5–2.5 mg/L. The dose-response relationship between the fluoride concentration in drinking water and the prevalence of dental caries was a typical U-shaped curve.

In our 2013 investigation, we only recruited 8–10 year-old-children, who born after the drinking water source changed to having a low level of fluoride. We compared the dental caries results for 2002 and 2013 for 8–10 year-old-children in the two villages. The prevalence of dental caries in Wamiao village was higher in 2013 than it was in 2002 but the difference was not significant ($\chi^2$=0.649, p=0.408). In Xinhuai village, the prevalence of dental caries was lower in 2013 than it was in 2002 but again the difference was not significant ($\chi^2$=0.702, p=0.402, Table 4). The level of dental caries in 2013 in these two village were at the low (DMFT range at 12 yr 1.2–2.6) or very low (DMFT range at 12 yr 0.0–1.1) levels according to the dental caries criteria for countries published by WHO in 1995.[13]

**Table 4.** The prevalence of dental caries (DC) and decayed, missing, and filled permanent teeth (DMFT) in different age groups in the 2002 and 2013 investigations in the Wamiao and Xinhuai villages

| Village and date | Age (years) | No. of children | DC (%) | DMFT |
|---|---|---|---|---|
| Wamiao village 2002 | 8 | 20 | 65.00 | 1.45 |
| | 9 | 16 | 43.75 | 1.06 |
| | 10 | 19 | 47.37 | 1.00 |
| | Total | 55 | 52.72 | 1.18 |
| Wamiao village 2013 | 8 | 28 | 71.43 | 2.25 |
| | 9 | 23 | 60.87 | 1.48 |
| | 10 | 17 | 52.94 | 1.00 |
| | Total | 68 | 63.24* | 1.68† |
| Xinhuai village 2002 | 8 | 39 | 76.92 | 2.85 |
| | 9 | 46 | 73.91 | 2.46 |
| | 10 | 31 | 74.19 | 2.26 |
| | Total | 116 | 75.00 | 2.53 |
| Xinhuai village 2013 | 8 | 19 | 73.68 | 2.47 |
| | 9 | 23 | 69.56 | 2.39 |
| | 10 | 23 | 60.87 | 1.61 |
| | Total | 65 | 69.23* | 2.14† |

Comparing the results of the 2013 investigation with the 2002 investigation, in both Wamiao and Xinhuai villages, there were no significant differences in the prevalence of either dental caries (DC) or in decayed, missing, and filled permanent teeth (DMFT): $^{*}\chi^2 = 0.649$, p = 0.702; $^{\dagger}\chi^2 = 0.408$, p = 0.402.

## DISCUSSION

In 1979, when endemic fluorosis was identified as an important endemic disease which must be controlled, the First National Congress of Experts in the Control of Endemic Fluorosis was convened in Shanxi Province, China, in order to evaluate the experiences of all the provinces and municipalities in controlling fluorosis. Meanwhile the guide-lines for the control of endemic fluorosis in China at a national level were drawn up and the National Committee of Experts in Endemic Fluorosis Control was also established.[14] Defluoridation, to improve the quality of drinking water according to the “National Standards for Drinking Water Quality,” was the fundamental measure for the control of drinking-water-borne endemic fluorosis.[15] On the basis of the accumulated experience, the suggestion was put forward that programmes for improving the quality of drinking water through defluoridation should take account of the local conditions in the endemic fluorosis areas, i.e., where water containing a low concentration of fluoride existed in a deep underground layer, a deep well should be drilled; where water containing a low concentration of fluoride existed on the surface, this surface water should be used for drinking water purposes. By the end of 2010, 81.89% of the villages with drinking-water-borne endemic fluorosis had changed their water sources by using drinking water with low fluoride levels, and changes to 100% of the endemic fluorosis villages in China are expected to be completed in 2015.[3,4] In Wamiao village, two deep wells, over 150 m in depth, were drilled at the beginning of 2003 to improve the quality of drinking water and provided water with a fluoride level lower than 1 mg/L.

There are a large number of research reports, from many countries in the world, on fluoride and its adverse effects. In China, key monitoring for endemic fluorosis has been carried out from 1991, and covers 25 provinces, municipalities, and autonomous regions.[16] The monitoring includes the operational state of the defluoridation projects, the water concentrations of the fluoride, and the prevalence of dental and skeletal fluorosis.[16] The "Control Criteria for Endemic Fluorosis", the "National Standard of the People's Republic of China" (GB17017-1997), was issued in 1997 and revised in 2010.[17,18] However, the key monitoring and the national standard did not include the urinary fluoride concentrations, the prevalence of dental caries, or a comparison of the dental indicators of dental caries, dental fluorosis, and defect dental fluorosis, before and after the water sources changed.

In this study, we compared the exposure and effect indicators in the severe drinking-water-borne endemic fluorosis village of Wamiao, before and after the implementation of defluoridation with new water sources from two deep wells, and we selected the control village of Xinhuai in a non-endemic fluorosis area. When the fluoride level in the drinking water decreased in Wamiao the level of fluoride in the children's urine also decreased significantly, and in the 2013 investigation no significant difference was present in the urinary fluoride levels of the children in the two villages.

Before the use of low fluoride drinking water began in Wamiao in 2003, significant dose-response relationships were present between the concentration of fluoride in water from the household shallow wells in Wamiao and Xinhuai and the prevalence of dental fluorosis and, especially, defect dental fluorosis (Figures 1 and 2). The prevalence of dental fluorosis in Wamiao village in 2002 investigation was consistent with previous reports from endemic areas in other countries (76%–100%). In Rift Valley of Ethiopia, with a drinking water fluoride level of 0.3–2.2 mg/L, the prevalence of dental fluorosis was 91.8%.[19] Manji F et al. reported that in a rural area of Kenya, with a water fluoride level of 2 mg/L, the prevalence of dental fluorosis is 100%.[20,21] In Andhra Pradesh, India, with a water fluoride level of 0.7–4 mg/L, the prevalence of dental fluorosis was 100%.[22] In the present study with the 2002 results for the two villages combined, when the fluoride level was approximately 2.45 mg/L, the prevalence of dental fluorosis was 93.04% (Figure 1). The prevalence of defect dental fluorosis had a significant linear dose-response relationship with the fluoride level in household shallow wells in the villages suggesting that the prevalence of defect dental fluorosis may be better than the prevalence of dental fluorosis when analysing the dose-response relationship (Figure 2). After 10 years use of low-fluoride drinking water in Wamiao, from 2003 to 2013, the prevalence of dental fluorosis in Wamiao was significantly decreased and was not significantly different from Xinhuai village (Table 4).

Fluoridation of public water supplies has been one of the most popular approaches for reducing the prevalence of dental caries.[23] It has been widely considered to be an effective method for the prevention of caries at a concentration of <1 mg/L of fluoride, but the excessive consumption of water, especially when containing >1.5 mg F/L, can result in adverse effects including the development of fluorosis in bones and teeth.[24-26] However, epidemiological studies have revealed concerning results regarding the relationship of dental caries and dental fluorosis.[19,25-28] In our 2002 investigation, when we divided the children, according to the drinking water fluoride concentration in their household shallow wells, a U-shaped dose-relationship was present between the water fluoride concentration and the prevalence of dental caries and the DFMT. In other words, when the concentration of fluoride in drinking water was at a relatively level range, the prevalence of dental caries decreased as the concentration of fluoride increased but when the fluoride concentration increased beyond this range the prevalence of dental caries also increased. It was found that the teeth with moderate and severe fluorosis had more dental caries than teeth with no or mild fluorosis.[27,29] In our 2013 investigation, after 10 years of defluoridation, the prevalence of dental caries was increased slightly in Wamiao village and decreased slightly in Xinhuai village, but no significant difference in the prevalence of dental caries was found between the 2002 and 2013 investigations (Table 4). According the WHO criteria for comparing dental caries in countries, the DMFT in our 2002 investigation were very low in Wamiao and low in Xinhuai, while in our 2013 investigation they were very low or low in Wamiao and low in Xinhuai.[13]

As previously reported and confirmed by the findings of this survey, in our 2002 investigation we collected water samples from the household shallow wells for all the children and measured the fluoride concentration.[6,7] We found that the drinking water was the main source of fluoride intake in this study area,[6,7] consistent with the results of Wang's survey of Jiangsu Province.[30]

The limitations of our 2013 investigation are: (i) the sample size was relatively small, being 68 children in Wamiao and 65 in Xinhuai village, compared to the numbers in our 2002 investigation, 236 in Wamiao and 290 in Xinhuai, and (ii) we did not measure the intake of fluoride from sources other than drinking water, such as the food, air, and toothpaste, as we did earlier in our 2002 investigation.

The preventive effect of fluoride in drinking water for dental caries needs to be further studied, especially with consideration of other adverse effects, apart from dental fluorosis, such as neurotoxicity, other sources of systemic fluoride including food, dental products, drugs and industrial emissions, and evidence that it is the topical action of fluoride with inhibition of demineralization, enhancement of remineralization, and the inhibition of bacterial action in dental plaque, rather than its systemic effects, that is important in preventing dental caries in both adults and children.[31-34]

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