SERUM FLUORIDE AND DENTAL FLUOROSIS IN TWO VILLAGES IN CHINA

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SUMMARY: An epidemiological investigation was conducted to determine the relationship between dental fluorosis and serum fluoride (measured by a minitype fluoride ion selective electrode) in the children aged 8-13 years in the Jiangsu Province villages of Wamiao (water fluoride: 2.45±0.80 mg/L; range: 0.57–4.50 mg/L) and Xinhuai (water fluoride: 0.36±0.15 mg/L; range: 0.18–0.76 mg/L). When divided into five groupings, higher serum fluoride levels were significantly associated with higher prevalences of dental fluorosis and the more severe defect dental fluorosis, which included significant dose-response relationships between serum fluoride levels and these two degrees of dental fluorosis (regression equations: Y = 1025.512X – 20.005, R^2 = 0.830; and Y = 614.656X – 16.922, R^2 = 0.920). Gender related differences in serum fluoride levels and the prevalence of dental fluorosis were found in both village, but not age related differences. There was also a significant positive relationship between the levels of fluoride in drinking water and the levels of fluoride in serum (Pearson correlation coefficient = 0.860, p<0.001). The survey findings indicated that drinking water is the main source of fluoride intake in Wamiao and Xinhuai, along with a significant positive dose-response relationship between serum fluoride and dental fluorosis.

Keywords: China; Dental fluorosis; Fluoride in serum; Fluoride in water; Jiangsu Province; Wamiao village; Xinhuai village.

INTRODUCTION

Serum fluoride concentration is recognized as a good indicator of fluoride exposure and provides useful data for endemic fluorosis control and prevention.[1-4] Some researchers have reported a relationship between fluoride in serum and the degree of fluorosis.[5-12] However, the results of these studies, at least in China, are not consistent. Two investigations indicated that there were significant differences in serum fluoride levels between endemic and nonendemic fluorosis areas.[6-7] On the other hand, Fan and co-workers[7] and Li *et al*[12] found significant differences between serum fluoride levels in skeletal fluorosis cases and non-skeletal fluorosis cases in an endemic fluorosis area. Song *et al*[6] also discovered that as the symptoms of skeletal fluorosis became more severe serum fluoride levels increased, whereas Wang *et al*[8] found they did not.

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Since the relationship between fluoride levels in serum and the prevalence of dental fluorosis has had only limited study, the present investigation was undertaken to examine levels of fluoride in serum and drinking water and their relationship to the prevalence and severity of dental fluorosis.

MATERIALS AND METHODS

Two villages (Wamiao and Xinhuai), 64 km apart, near Lake Hongze, about 215 km inland from the coast and about 730 km southeast of Beijing, were selected for study in Sihong County, Jiangsu Province, People's Republic of China. Wamiao village (drinking water fluoride mean and range in mg/L: 2.45±0.80, 0.57–4.50), in northeast Sihong County, about 32 km northeast of Sihong and 40 km southwest of Huaiyin, is located in a severe endemic fluorosis area, while Xinhuai village (drinking water fluoride mean and range in mg/L. 0.36±0.15, 0.18–0.76), in the southwest part of Sihong County, about 32 km southwest of Sihong and 100 km southwest of Huaiyin, is in a nonendemic fluorosis area. Neither village has fluoride pollution from burning coal or other industrial sources. None of the residents drink brick tea.

The study was conducted between September 2002 and June 2003. All the children, aged 8–13 years old, were investigated in both village primary schools. A questionnaire, completed with the assistance of the parents, was used to collect information on personal characteristics, exposure history to fluoride, medical history, family socioeconomic status, and lifestyle. Children who had been absent from either village for two years or longer, or who had a history of immigration were excluded. In Wamiao 97.12% of the children (236 out of 243) were selected, while in Xinhuai 95.08% were included (290 out of 305). Children in the two villages, considered as a whole, were divided into five subgroups according to the levels of fluoride in their serum: <0.030 mg/L (group A), 0.030–0.059 mg/L (group B), 0.060–0.089 mg/L (group C), 0.090–0.119 mg/L (group D), ≥0.120 mg/L (group E).

Fluoride in serum was determined with a minitype fluoride ion selective electrode and in drinking water with a standard fluoride ion selective electrode.[2,13] Fasting venous blood samples (2–2.5 mL) were collected and preserved in clean plastic centrifuge tubes, which were immediately centrifuged for 10 min at 3000 rpm. Serum was removed to other clean plastic tubes and kept in a refrigerator at 4ºC and subsequently analyzed within one week. The linear ranges of the method for the serum fluoride measurements were 0.02–0.50 mg/L, r = 0.9999, b = 57.8. The lower test limit of this method was 0.012–0.013 mg/L, and the coefficient of variation ranged from 0.99% to 4.72%.[14,15] The drinking water samples, which were collected from the household shallow wells in each child's family, were kept in clean plastic bottles and analyzed within one week.

A dentist and a specialist in endemic fluorosis control and prevention examined the children for dental fluorosis with a mouth mirror, forceps, and a probe under natural light. Dean's classification and the Chinese "Clinical diagnostic standard for dental fluorosis" (WS/T208-2001)[16] were used for diagnosing dental fluorosis. The six grades of Dean's classification scale for dental fluorosis are: none (normal enamel) (0), suspected or questionable (0.5), very mild (1), mild (2), moderate (3), and severe (4). In the WS/T208-2001 scheme, dental fluorosis is divided into eight grades. Grades 5 (which has a small amount pitting on the surface of enamel) and above were considered "defect dental fluorosis." Statistical analysis of the prevalence of dental fluorosis was made according to the rates of DF% (DF, dental fluorosis by Dean's classification) and the rates of DDF% (DDF, defect dental fluorosis by the WS/T208-2001) scheme. DDF included some "moderate" dental fluorosis (grade 3) and all "severe" dental fluorosis (grade 4) diagnosed by Dean's criteria.

$$\text{Prevalence of dental fluorosis (DF\%)} = \frac{\text{No. of children with very mild dental fluorosis or higher}}{\text{No. of children investigated}} \times 100$$

$$\text{Prevalence of defect dental fluorosis (DDF\%)} = \frac{\text{No. of children with defect dental fluorosis}}{\text{No. of children investigated}} \times 100$$

The data were analyzed using SAS and Excel 2000.

RESULTS

The two villages are situated in isolated, poverty-stricken areas with low economic development and a relative lack of communication with the outside world resulting in poor living conditions for the majority of the residents, especially the elderly and children. There are no significant differences between Wamiao village and Xinhuai village in diet, medical care, socio-economic standard, lifestyle, communication, climate, etc.

As shown in Table 1 and Table 2, significant differences were found between Wamiao and Xinhuai in drinking water fluoride, serum fluoride in the children, and the prevalences of dental fluorosis and defect dental fluorosis.

Table 1. Fluoride in drinking water and serum in two villages

Village	No. of samples	F^- in drinking water (mg/L)		F^- in serum (mg/L)	
		Mean ± SD	Range	Mean ± SD	Range
Wamiao	236	2.45 ± 0.80	0.57 – 4.50	0.081 ± 0.019	0.040 – 0.160
Xinhuai	290	0.36 ± 0.15	0.18 – 0.76	0.041 ± 0.009*	0.021 – 0.094

*p<0.001, compared with Wamiao village.

Table 2. Prevalence of DF and DDF in children aged 8 – 13 in two villages

Village	No. of children	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
Wamiao	236	209	92	88.56	38.98
Xinhuai	290	13	0	4.48*	0*

*p<0.001, compared with Wamiao village.

Data from the 526 children in the two villages were combined and considered as a whole to make five subgroups as shown in Table 3, from which it can be seen that when the serum fluoride increased, the prevalence of dental fluorosis and defect dental fluorosis also increased. The dose-response relationship between serum fluoride in the children and the prevalence of DF and of DDF was significant. The regression equation between serum fluoride and DF is $Y = 1025.512X – 20.005$ ($R^2 = 0.830$), and between serum fluoride and DDF is $Y = 614.656X – 16.922$ ($R^2 = 0.920$).

Table 3. Relationship between serum fluoride and dental fluorosis

Groups	No. of samples & children	F^- in serum (mg/L)	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
A	18	0.027 ± 0.002	0	0	0	0
B	280	0.042 ± 0.007	23	2	8.20	0.71
C	169	0.074 ± 0.008	145	60	85.80	35.50
D	47	0.100 ± 0.009	44	25	93.62	53.19
E	12	0.135 ± 0.010	12	7	100.00	58.33

In Xinhuai but not in Wamiao, a significantly higher serum fluoride level was found in boys than in girls ($t = 2.136$, $p = 0.033$). However, the serum fluoride levels in both boys and girls living in Wamiao were about double of the levels in Xinhuai. There were significant differences between boys and girls in the prevalence of DF in both villages ($p<0.05$). In Wamiao the prevalence of DDF of boys was higher than that of girls, but the difference was not significant. The details of these comparisons are shown in Tables 4 and 5.

Table 4. Prevalence of DF and DDF vs. serum fluoride by gender in Wamiao village

Gender	No. of children	F^- in serum (mg/L) Mean ± SD	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
Boys	130	0.0798 ± 0.0170	122	56	93.85	43.08
Girls	106	0.0818 ± 0.0217	87	36	82.08*	33.96

*$p < 0.05$, compared with boys.

Table 5. Prevalence of DF and DDF vs. serum fluoride by gender in Xinhuai village

Gender	No. of children	F^- in serum (mg/L) Mean ± SD	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
Boys	159	0.0425 ± 0.0087	11	0	6.92	0
Girls	131	0.0402 ± 0.0092	2	0	1.53*	0

*$p < 0.05$, compared with boys.

As shown in Tables 6 and 7, there were no significant age-dependent differences and trends in serum fluoride levels and the prevalence of either DF or DDF in either village. However, in each age group the level of fluoride in serum and the prevalence of DF and DDF in Wamiao were considerably higher than in Xinhuai.

Table 6. Prevalence of DF, DDF, and fluoride in serum by age in Wamiao village

Age	No. of children	F^- in serum (mg/L) Mean ± SD	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
8	20	0.0841 ± 0.0278	18	5	90.00	25.00
9	16	0.0739 ± 0.0131	13	4	81.25	25.00
10	19	0.0875 ± 0.0260	18	7	94.74	36.84
11	39	0.0800 ± 0.0172	35	19	89.74	48.72
12	69	0.0796 ± 0.0184	60	23	86.96	33.33
13	73	0.0809 ± 0.0172	65	34	89.04	46.58

Table 7. Prevalence of DF, DDF, and fluoride in serum by age in Xinhuai village

Age	No. of children	F^- in serum (mg/L) Mean ± SD	No. with DF	No. with DDF	Prevalence of DF (%)	Prevalence of DDF (%)
8	39	0.0422 ± 0.0091	1	0	2.56	0
9	46	0.0423 ± 0.0091	1	0	2.17	0
10	31	0.0410 ± 0.0087	1	0	3.23	0
11	60	0.0406 ± 0.0072	5	0	8.33	0
12	61	0.0427 ± 0.0115	4	0	6.56	0
13	53	0.0399 ± 0.0071	1	0	1.89	0

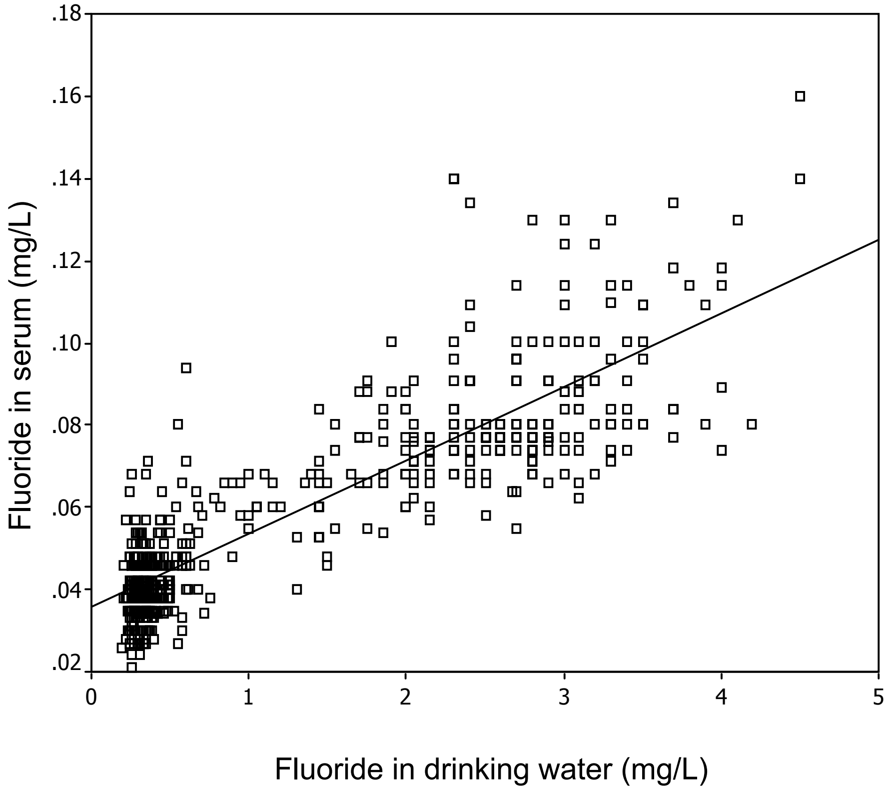

Figure 1. Correlation between fluoride in drinking water and serum. (Pearson correlation =0.860, $p < 0.001$).

A significant positive relationship was also found between the level of fluoride in the drinking water and fluoride in the serum of the children. As the fluoride level in the drinking water increased, so did the serum fluoride level (Pearson correlation coefficient = 0.860, $p<0.001$).

The regression equation between the levels of fluoride in drinking water and serum was $Y = 0.018X + 0.036$ ($R^2 = 0.740$). The results suggest that fluoride in drinking water is the main source of fluoride intake in Wamiao and Xinhuai (Figure 1).

In regard to fluoride intake over time, almost every home in both Xinhuai and Wamiao village has a shallow household well in their yard, as illustrated in Figure 2. These wells provide water for drinking and washing for the home. When a shallow well fails, the peasants dig another one nearby in their yard. Since the inhabitants seldom move from their residence to another area, the children's exposure to fluoride in drinking water can be estimated from the length of time the household shallow well was in use. As shown in Table 8, the mean exposure to fluoride in drinking water was about three years longer in Wamaio than in Xinhai.

Figure 2. A typical household shallow well.

Table 8. Basic information about the household shallow wells in Xinhaui and Wamiao villages

Village	No. of children	Years of use of household shallow wells	
		Mean ± SD	Range
Xinhuai	290	5.16 ± 3.12	1 – 16
Wamiao	236	8.27 ± 3.02	1 – 15

DISCUSSION

Stepwise multiple regression analysis indicates that there are significant positive relationships between the degree of endemic fluorosis and the level of fluoride in drinking water. The regression equations between levels of fluoride in the drinking water and prevalences of dental fluorosis or skeletal fluorosis are $Y = 24.741X – 4.604$ ($r = 0.729$, $p<0.01$) and $Y = 6.55X – 0.48$ ($r = 0.96$, $p<0.001$), respectively. However, no correlation has been found with the fluoride levels in grains (flour, rice, and meal) and vegetables or soil in Jiangsu Province, China.[17-18] Consequently, in this region endemic fluorosis is caused mainly by fluoride in the drinking water. This finding is consistent with the present study indicating that fluoride in drinking water was the main source of fluoride intake.

In China, villages have had land reform beginning early in the 1980s. Peasants have contracted land from the local government for about 30 years, with the longest term of a valid contract being about 50 years. Hence they and their children hardly ever move from one residential site to another one. As shown in Table 8, the mean exposure time to fluoride in drinking water of the children aged 8-13 was a little over eight years in Wamiao village and a little over five years in Xinhuai village. It is likely therefore that exposure to fluoride in drinking water occurred during permanent tooth formation and enamel maturation, and thus the present findings of fluoride levels in the drinking water and serum probably reflect the fluoride exposure of the children during infancy and early childhood.

As found here, there were significant dose-response relationships between serum fluoride levels and the prevalence of dental fluorosis and defect dental fluorosis, which are consistent with the findings of Song *et al.*[8] Some researchers have reported no gender related differences in serum fluoride in healthy adults.[12, 19-21] The results of the present study, however, revealed that the mean serum fluoride level in boys 8 to 13 years of age was significantly higher than in girls ($p<0.05$) in the low-fluoride village of Xinhuai, whereas it was not in the high-fluoride village of Wamiao.

We also found significant gender related differences in the prevalence of dental fluorosis in both villages, which are consistent with the findings of Sun *et al*[22] and Li *et al*,[23] but not those of Cao *et al*[24] and Fan *et al.*[25] Possible reasons why there were gender related differences in serum fluoride levels and the prevalence of dental fluorosis among the children in both villages might include a greater physical activity and total intake of drinking water by the boys than the girls, leading to a higher level of fluoride intake by the boys than the girls. However, the exact reasons require further study. Finally, it should be noted that no age related differences in serum fluoride levels and the prevalence of either DF or DDF were found in the children in either village.

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