SERUM FLUORIDE AND SKELETAL FLUOROSIS IN TWO VILLAGES IN JIANGSU PROVINCE, CHINA

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SUMMARY: Serum fluoride in relation to the prevalence of skeletal fluorosis was investigated in two villages in Jiangsu Province, China. In the high-fluoride village of Wamiao, 132 adults (average age 52.36 years; water fluoride 2.18±0.86mg/L; range 0.85–4.50mg/L) were surveyed. In the low-fluoride village of Xinhuai, 35 adults (average age 48.11 years; water fluoride 0.37±0.09 mg/L; range 0.21–0.55mg/L) were surveyed. Subjects were recruited by sampling according to the fluoride content of the drinking water in their household wells. When the subjects were divided into five subgroups according to their serum fluoride concentration, higher serum fluoride concentration was strongly associated with a higher prevalence of skeletal fluorosis in the form of a significant positive dose-response relationship (regression equation: $Y = -27.29+890.42X-223.20X^2$). In Wamiao village a significant difference was also found between serum fluoride concentrations in 41 subjects with X-ray detectable skeletal fluorosis and in 91 subjects without X-ray detectable skeletal fluorosis. Gender related differences in serum fluoride concentration, household well water fluoride, and the prevalence of skeletal fluorosis were not found in the subjects in Wamiao village. These findings indicate that serum fluoride concentrations have a significant positive dose-response relationship with the prevalence of skeletal fluorosis in an endemic fluorosis area associated with high-fluoride drinking water.

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

INTRODUCTION

Serum fluoride concentration is recognized as a good indicator of fluoride exposure and provides an important basis for endemic fluorosis control and prevention.[1-5] Findings of Fan et al.[6] and of Li et al.[7] indicated a significant difference in serum fluoride between cases of skeletal fluorosis and those without X-ray detectable skeletal fluorosis in an endemic fluorosis area. Song et al.[8] also found that the severity of skeletal fluorosis is directly related to elevated serum fluoride levels, although studies by Wang et al.[9] suggested otherwise. In general, data on both serum fluoride concentration and the prevalence of skeletal fluorosis in adults are not often reported. In the present study the levels of fluoride in serum and drinking water and the prevalence of skeletal fluorosis in two villages in China are examined and the relationships are analyzed.

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MATERIALS AND METHODS

Two villages (Wamiao and Xinhuai), 64 km apart, near Lake Hongze, 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) is located in a severe endemic fluorosis area, whereas Xinhuai village (drinking water fluoride mean and range in mg/L: 0.36±0.15, 0.18–0.76) is in a non-endemic fluorosis area. Neither village has fluoride pollution from burning coal or other industrial sources. No residents drink brick tea.

The study was conducted from September 2002 to September 2003. A total of 167 adults aged 36–78 were recruited for study—132 in Wamiao village and 35 in Xinhuai village. Subjects were selected from the two villages on the basis of the fluoride concentration in the drinking water obtained from their shallow household wells. A questionnaire was used to collect information on personal characteristics, history of exposure to fluoride, medical history, family socioeconomic status, and lifestyle. Those who had been absent from the village for two years or longer and women who had married from other areas less than 20 year previous were excluded. The average age of the subjects in Wamiao was 52.36 years and 48.11 years in Xinhuai. The male:female ratio was 1.28:1 in Wamiao and 1.50:1 in Xinhuai.

For the diagnosis of skeletal fluorosis, X-rays were taken of the right forearm (upright and lateral position) and pelvis (upright position).

Fluoride in serum was measured with a minitype fluoride ion selective electrode, and drinking water fluoride was determined with a standard fluoride ion selective electrode.[2,10] Fasting venous blood samples (2–2.5 mL) were collected and preserved in clean plastic centrifuge tubes and centrifuged immediately at 3000 rpm for 10 min. The serum was removed to other clean plastic tubes and kept in a refrigerator and subsequently analyzed within one week. The linear ranges of the method for serum fluoride measure 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%.[11,12] The drinking water samples, which were collected from the shallow household wells in each subject's family residence, were kept in clean plastic bottles and analyzed within one week.

Skeletal fluorosis was diagnosed by endemic disease control and prevention specialists and by radiographic diagnosis specialists according the Chinese National Standard and the Chinese National Department Standard: "Diagnosis of clinical classification for endemic skeletal fluorosis" (GB 16396–1996)[13] and "Radio diagnosis of skeletal fluorosis" (WS 192–1999).[14] In GB 16396 skeletal fluorosis is classified into three grades: Grade I (mild): X-rays indicate signs of skeletal fluorosis but without clinical symptoms of limited joint motion and joint deformation. Grade II (moderate): these cases exhibit symptoms or clinical signs of joint ache, stiffness, malfunction, or deformation, but the subjects are able to do housework. Grade III (severe): these cases have severe symptoms or clinical signs of lateral curvature, bent waist and back, and loss of the ability to work, often accompanied by non-skeletal damage, such as parathyroid gland dysfunction, hypothyroidism, and chronic renal malfunction, etc.

RESULTS

The serum fluoride concentrations of the subjects and the drinking water fluoride levels in their household shallow wells are shown in Tables 1 and 2. There are significant differences between Wamiao and Xinhuai village in the serum fluoride and the drinking water fluoride concentrations.

Table 1. Fluoride in blood serum of subjects in Wamiao and Xinhuai villages

Village	No. of subjects	Fluoride in serum (mg/L)	
		Mean±SD	Range
Wamiao	132	0.064±0.020	0.034-0.160
Xinhuai	35	0.045±0.003*	0.038-0.054

* $p<0.001$ compared with Wamiao village.

Table 2. Fluoride in drinking water in the household shallow wells in Wamiao and Xinhuai villages

Village	No. of subjects	Fluoride in water (mg/L)	
		Mean±SD	Range
Wamiao	132	2.18±0.86	0.85-4.50
Xinhuai	35	0.37±0.09*	0.21-0.55

* $p<0.001$ compared with Wamiao village.

As shown in Table 3, there is also a highly significant difference between the two villages in the prevalence of skeletal fluorosis. In the low-fluoride drinking water village of Xinhuai there were no cases of skeletal fluorosis. In high-fluoride Wamiao, of the 41 cases of skeletal fluorosis, 39 were Grade I, two cases were Grade II, but none were Grade III.

Table 3. Prevalence of skeletal fluorosis in Wamiao and Xinhuai villages

Village	No. of subjects	No. of cases	Prevalence of skeletal fluorosis (%)
Wamiao	132	41	31.06
Xinhuai	35	0	0*

* $p<0.001$ compared with Wamiao village.

As shown in the Figure, in one of the skeletal fluorosis cases the X-ray film of forearm bone showed increased bone mineral density and a fish-scale shape proliferation on the periosteum of the ulna and radius.

Figure. X-ray of forearm of a grade I skeletal fluorosis case.

The subjects from the two villages were divided into five subgroups according their serum fluoride: 0.040 mg/L (group A), 0.041–0.060 mg/L (group B), 0.061–0.080 mg/L (group C), 0.081–0.100 mg/L (group D), and >0.100 mg/L (group E). As shown in Table 4 there is a significant positive dose-response relationship between the level of serum fluoride and the prevalence of skeletal fluorosis ($r = 0.95$; regression equation $Y = -27.29 + 890.42X - 223.20X^2$). The average ages show a tendency to increase along with the increase in serum fluoride. Groups A and B are significantly different when compared to group D.

Table 4. Relationship between serum fluoride and skeletal fluorosis

Group	No. of subjects & serum samples	Average age Mean±SD (years)	Serum fluoride Mean±SD (mg/L)	No. of subjects with skeletal fluorosis	Prevalence of skeletal fluorosis (%)
A	15	47.67±10.49*	0.039±0.002	1	6.67
B	90	49.36±8.89*	0.050±0.006†	11	12.22†
C	39	54.36±9.88	0.067±0.006†	17	43.59†
D	15	58.73±9.69	0.089±0.007†	6	40.00†
E	8	54.75±8.14	0.115±0.020†	6	75.00†

* $p<0.01$ compared with group D; †$p<0.001$ compared with group A.

The prevalence of skeletal fluorosis in males and females in Wamiao village is shown in Table 5. There is a significant 3.69-year average age difference between males and females ($p = 0.045$). The prevalence of skeletal fluorosis was not significantly different, however, ($p = 0.26$), even though it was higher among females than males. The average household well water fluoride and serum fluoride concentrations of the male subjects in Wamiao village were 2.12±0.83 and 0.066±0.018 mg/L, respectively, and 2.26±0.89 and 0.062±0.022 mg/L in females. These differences are not statistically significant.

Table 5. Prevalence of skeletal fluorosis in males and females in Wamiao village

Gender	No. of subjects	Average age Mean±SD (years)	No. of subjects with skeletal fluorosis	Prevalence of skeletal fluorosis (%)
Male	74	53.64±11.56	20	27.03
Female	58	49.95±8.70*	21	36.21

*$p<0.045$ compared with males.

As seen in Table 6, the average age difference of 6.17 years for males and females with skeletal fluorosis in Wamiao village was significant ($p = 0.040$). There was, however, no significant difference in serum fluoride and household drinking water fluoride between the two sexes.

Table 6. Average ages, serum fluoride, and household drinking water fluoride between males and females with skeletal fluorosis in Wamiao village

Gender	No. of subjects with skeletal fluorosis	Average age Mean±SD (years)	Serum fluoride Mean±SD (mg/L)	Drinking water fluoride Mean±SD (mg/L)
Male	20	57.65±10.43	0.076±0.019	2.99±0.076
Female	21	51.48±8.05*	0.073±0.029	2.98±0.065

*$p<0.040$ compared with males.

Finally, as seen in Table 7, there are significant differences between the number of subjects with skeletal fluorosis and the number without skeletal fluorosis in relation to serum fluoride concentration and household well drinking water fluoride in Wamiao village.

Table 7. Serum fluoride and drinking water fluoride of subjects skeletal fluorosis and without skeletal fluorosis in Wamiao village

Group	No. of subjects	Serum fluoride Mean±SD (mg/L)	Drinking water fluoride Mean±SD (mg/L)
Without skeletal fluorosis	91	0.060±0.016	1.82±0.65
With skeletal fluorosis	41	0.074±0.024*	2.99±0.70*

* $p<0.001$ compared with subjects without skeletal fluorosis.

DISCUSSION

In Jiangsu Province, the regression equation between the concentration of fluoride in the drinking water and the prevalence of skeletal fluorosis was found by Wang et al. to be $Y = 6.55X - 0.48$, with an estimated 300,000 cases of skeletal fluorosis.[15] The villages of Wamiao and Xinhuai are located in an isolated rural area of the province. Low income and a relative lack of communication restrict emigration of the residents, especially the elderly. The results of this investigation indicated that the ratio of residents, aged 40 years or over, who had been absent from their hometown for more than two years (accumulated time), was only 1.00% in Wamiao, and 1.20% in Xinhuai. As previously reported and confirmed by the findings of this survey, the drinking water was the main source of fluoride intake in this study area.[16] The fluoride exposure history of the subjects in this study is clear and is consistent with the results of Wang's survey of Jiangsu Province.[15]

Skeletal fluorosis is the end result of long-term exposure to chronic fluoride intoxication, and is graded according to the appearance of bone on X-ray.[1] At 3.2 ppm, 3.7 ppm, and 4.0 ppm water F concentration in villages in India, the highest prevalence of skeletal fluorosis was 39.2, 32.8, and 36.6%, respectively.[17] Crippling fluorosis was found at and above 2.8 ppm F.[17] In the present study, the prevalence of skeletal fluorosis is higher than that reported by Choubisa,[17] but crippling fluorosis was not found. In the present study there was no gender difference between males and females in the prevalence of skeletal fluorosis. This finding is consistent with those of Choubisa. A study by Watanabe et al. found that the prevalence of skeletal fluorosis was significantly higher for males in a moderately polluted area, but not in a severely polluted area.[18] This matter clearly requires further study.

The present study demonstrates a positive significant dose-response relationship between the serum fluoride concentrations and the prevalence of skeletal fluorosis. The correlation coefficient was 0.95 by the model of quadratic fit. In the study of Li et al.,[19] the correlation coefficient between the serum fluoride levels and the prevalence of skeletal fluorosis was 0.88. A highly significant difference between the number of subjects with skeletal fluorosis and those without skeletal fluorosis in relation to their serum fluoride concentration and the household well drinking water fluoride was found in this study. These findings are consistent with those reported by Fan et al.[6] and by Li et al. [7]

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REFERENCES

1 Wan G.M, Chen Z, Zhang Lihong, et al. Fluorine in serum or plasma. Chin J Ctrl Endem Dis 2000;15(2):90-2. [in Chinese]

2 National Department Standard of P. R. China. Determination for fluoride in serum – Ion selective electrode method. (WS/T 212-2001).

3 Parkins FM, Tianoff N, Moutinho M, et al. Relationship of human plasma fluoride and bone fluoride to age. Calcif Tissue Res 1974;16:335-8.

4 Kono K, Yoshida Y, Watanabe M, et al. Urine, serum and hair monitoring of hydrofluoric acid workers. Int Arch Occup Environ Health 1993;65:S95-8.

5 Kono K, Yoshida Y, Watanabe M, et al. Serum fluoride as an indicator of occupational hydrofluoric acid exposure. Int Arch Occup Environ Health 1992;64:343-6.

6 Fan SG, Wang T, Tang XL, et al. Relationship among the level of fluoride in indoor air, in serum and in saliva in the endemic fluorosis area caused by burning coal. Chin J Ctrl Endem Dis 1992;7(1):41. [in Chinese]

7 Li CS, Ke XD. Ionic, nonionic, and total fluoride in human serum. Fluoride 1990, 23(4):164-70.

8 Wang DS. Study on the relationship between the fluoride in drinking water and serum in endemic fluorosis and nonendemic fluorosis areas. Chinese Journal of Preventive Medicine 1986; 20(5):292. [in Chinese]

9 Song CF, Huang WY, Yan BW. Study on the relationship between the biochemical assay of blood and the patient's condition of skeletal fluorosis cases caused by burning coal. Chinese Journal of Endemiology 1995;14(5):291-293. [in Chinese]

10 National Standard of P. R. China. Standard examination method for drinking water. (GB 5750-1985).

11 Ye P, Liou Y, Bian JC. Verification and application of standard method for detecting serum fluoride. Chinese Journal of Endemiology 2001;20(2):140-2. [in Chinese]

12 Wan GM, Wang SZ, Xu CB, et al. Study on standard method for determination inorganic fluoride in serum. Chin J Ctrl Endem Dis 2000;15(5):266-9. [in Chinese]

13 National standard of P. R. China. Diagnosis of clinical classification for endemic skeletal fluorosis. (GB 16396-1996).

14 National department standard of PR China. Radio diagnosis of skeletal fluorosis (WS 192 – 1999).

15 Wang CS, Bu XH, Gu SD. The epidemiological study of endemic fluorosis in Huai-bei region, Jiangsu Province. Chinese Journal of Endemiology 1997; Suppl 12(4-A):10-3. [in Chinese]

16 Xiang QY, Liang YX, Chen BH, et al. Serum fluoride and dental fluorosis in two villages in China. Fluoride 2004;37(1):28-37.

17 Choubisa SL. Endemic fluorosis in Southern Rajasthan, India. Fluoride 2001;34(1):61-70.

18 Watanabe T, Kondo T, Asanuma S, Ando M, Tamaru K, Sakuragi, et al. Skeletal fluorosis from indoor burning of coal in Southwestern China. Fluoride 2000;33(3):135-9.

19 Li D, Yao Z, Li P, et al. Correlative study of serum fluoride levels, urine fluoride levels, prevalence of dental fluorosis and skeletal fluorosis in coal-burning type of endemic fluorosis area. Chinese Journal of Endemiology 1996;15(5):264-6. [in Chinese]

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