The association of environmental fluoride, trace elements and urine fluoride in adults living in endemic fluorosis villages in Henan Province

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Abstract: Objective To analyze the relationship of environmental fluoride level, urine fluoride concentration and trace elements in adults living in endemic fluorosis villages. Methods Fluoride concentrations in drinking water, vegetables, crops, soils and urine were determined using fluorine ion selective electrode method. The concentrations of the Calcium (Ca), magnesium (Mg) in drinking water and serum in adults were detected using the flames atom absorption method. Results The concentrations of fluoride in drinking water were 2.15±1.97mg/L, 0.46±0.05mg/L and 0.38±0.15mg/L in the endemic fluorosis villages (EFV), villages conducted defluoridation project (DFPV) and control villages (CV) respectively. The fluoride concentration in drinking water of EFV was significantly higher than that of CV and DFPV (P<0.05 respectively). The concentration of fluoride in the plough layer in the high fluoride village was higher compared with control villages (P<0.05). There were no statistical differences of fluoride concentrations in vegetables, grain and plow pan layer of soil among EFV, DFPV and CV (P>0.05). The concentrations of the fluoride in the urine were 2.50±1.50 mg/L, 1.42±0.97mg/L and 0.98±0.50 mg/L in adults from EFV, DFPV and CV respectively. There were statistical differences between any two of the three groups (P<0.05). There were no significant differences among the concentration of Ca^{2+} and Mg^{2+} in drinking water of EFV, DFPV and CV. There was negative correlation between blood Ca^{2+} and urine fluoride (r=-0.183, P=0.022). Conclusion Individuals who have higher urine fluoride level tend to have lower blood Ca^{2+} concentration.

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1 Introduction

Fluorosis is a public health problem in some areas resulting from long-term consumption of water with high fluoride (F^-) level. Most obvious sign of this disease is mottled enamel on the teeth of the local people (Oruc, 2008) Fluoride accumulates over time because of its long biological half-life in bone and this accumulation can cause skeletal fluorosis, a painful condition. Radiological reports of people who have fluoride toxicity show the bone to be affected by osteosclerosis osteopenia and/or osteomalacia(Tamer et al, 2007; Wang et al, 2007) Endemic fluorosis areas are still exist in many provinces in China such as Henan, Shanxi, Yunnan and so on though the prevalence of this disease has decreased considerably after controlling the fluoride ingestion by defluoridation project and improvement of stove. Fluoride, calcium (Ca), magnesium (Mg) and other trace elements play important roles in the occurrence and development of endemic fluorosis. In the study of endemic fluorosis, the distributions of some chemical elements in the internal and external environment and their relationships with fluorosis have attracted more and more attention in this field. Some researches in China about the relationship between fluoride and relative elements have made a great progression especially in the aspect that antagonistic or synergistic effect of trace elements to the toxic function of fluoride (Wang et al, 2007). Previous studies showed that dental fluorosis index would be decreased in children living in endemic fluorosis areas with higher calcium in drinking water compared to those areas with relatively lower calcium in drinking water even if their fluoride level in drinking water was similar (Ba and Cui, 1995). At present many studies focus on the relationship of environmental fluoride, urine fluoride and trace elements, but the results can not reach an agreement. Here, we conducted a cross sectional study in Henan province to investigate the association of environmental fluoride, trace elements and urine fluoride in adults living in endemic fluorosis villages.

2 Materials and Methods

2.1 Location and subjects:

A cross sectional study was conducted in six villages of Tongxu County in Henan province, China in 2011 by using simple cluster sampling method. It included three endemic fluorosis villages and three non-endemic fluorosis villages. Endemic fluorosis villages (EFV) were defined as villages with fluoride levels exceeding 1.0 mg/L (Chinese water quality standard) in drinking water. Two of the three villages were conducted defluoridation project (DFPV) of drinking water at the end of 2008. Non-endemic fluorosis villages were defined as control villages (CV) with fluoride levels of less than 1.0 mg/L in drinking water. There were no significant differences in the natural environment, socioeconomic status, life styles, dietary habits, and other demographic characteristics among the six villages. Local residents who were aged between 18 and 50 years for male, 18-48 for female and who were born and raised in the six villages were considered eligible for the study. By questionnaire and healthy physical examination, we excluded subjects who had received drug treatment in forms of bisphosphonates, calcitonin, fluoride, or hormone replacement therapy and⁄or who had hip fractures. A total of 894 participants met the inclusion criteria in this study composed of 287men and 607 women with the participation rate of 90.30% (894/990). Each subject provided two 5 ml fasting blood samples and 50 ml of instant urine sample. Blood was collected in red top vacuum tubes, and placed immediately on ice. After centrifugation, serum and white blood cells were separated and frozen at -70℃ for subsequent analyses. All procedures were approved by the Institutional Review Board at Zhengzhou University, China.

2.2 Detection of Fluoride levels

The fluoride concentrations of drinking water and each of the urine samples from each of the subjects were determined in duplicate using a fluoride ion selective electrode (Shanghai Exactitude Instrument Company, China) connected to a digital pH/mV meter. The fluoride concentrations of collected vegetables, crops, and soils were determined by the combination ion-selective fluoride electrode after adequate pre-treatment of the samples which included sample drying, weighing, grinding and so on. The dried vegetables were dissolved with 6 mL of 0.1 N HCl and then shaken in the constant temperature shaker for one hour. The supernatants were collected after centrifuging with 5000 rpm for 15 minutes and repeated this step with 6 ml deionized water. All the collected supernatants were moved into 25 ml volumetric flask and brought to volume by deionized water and TISAB Ⅰ (10:1 v/v). Fluoride concentration was measured in 50ml beaker with 25ml sample, 12.5ml TISAB Ⅰ and 12.5ml deionized water by using a combination fluoride electrode method. The final result was corrected for blank F concentrations assessed in parallel and determined through a standard addition method.

2.3 Detection of calcium and magnesium levels

The concentrations of calcium and magnesium in drinking water were determined by atomic absorption spectrophotometry (Hitachi 10 Z-5000, Japan) (Lu et al, 2008). The serum concentrations of calcium, magnesium were also measured by atomic absorption spectrophotometry after mixed-acid digestion with nitric acid and perchloric acid (4:1 v/v) The final result was corrected for blank value concentrations assessed in parallel and determined through a calibration curve method.

2.4 Statistical analysis

The data was analyzed by the SPSS software, version 12.0 (SPSS Inc, 2003). Continuous parameters between groups were compared with Student's t test, and continuous parameters among 3 or more groups were compared with One-way ANOVA if all the numeric materials belong to normal distribution. However, if the data didn't meet normal distribution, it should be transformed to achieve approximately normal distribution using logarithmically-transformed method, and the transformed values were used in data analyses. Pearson correlation analysis was made between drinking water fluoride and the trace elements. So did between urine fluoride and blood Ca, Mg. A P value <0.05 was considered statistically significant.

3 Results

3.1 The comparison of fluroride, Ca, and Mg concentrations in drinking water in different groups (Table 1)

The fluoride concentration in drinking water of EFV was significantly higher than those of CV and DFPV. ($P < 0.05$ respectively). No significant differences were observed between DFPV and CV (P>0.05). The concentrations of Ca^{2+} and Mg^{2+} in drinking water were also shown in table 1, from which we can see that there were no significant differences of Ca^{2+} concentrations among EFV, DFPV and CV, but the concentrations of Mg^{2+} in drinking water of any two groups have significant differences ($P<0.05$ respectively).

Table 1. The comparison of Ca, Mg and fluoride concentrations（mg/L, $\overline{\chi}\pm s$） of EFV, DFPV and CV in drinking water.

group	n	Ca^{2+}	Mg^{2+*}	Fluoride##
EFV	13	77.84±72.48	41.61±17.59	2.44±1.88
DFPV	8	44.98±4.57	6.10±1.46	0.36±0.30
CV	16	96.34±27.13	31.34±6.01	0.37±0.15
F		2.532	22.542	12.289
P		0.094	**0.000**	**0.000**

* There was significant difference between any two of the three groups.

The fluoride concentration in drinking water of EFV was significantly higher than those of CV and DFPV.

3.2 The comparison of fluoride in foods, vegetables and soils of different villages (Table 2)

The comparison of fluoride in crops, vegetables and soils in different investigated villages were shown in Table 2. Due to the irrigation water in DFPV is still the water which from the wells before defluoridation project; we merged the vegetables, grain and soil samples from EFV and DFPV as the samples of endemic fluorosis villages (EFV+DFPV). As we can see from it that there were no significant differences of fluoride concentration in vegetables, crops and plow pan layer of soil between EFV+DFPV and the CV (P ＞ 0.05), but the water-soluble fluorine and total fluorine in plough layer were higher in EFV+DFPV than those in CV (P<0.05).

Table 2. The comparison of fluoride in food, vegetable and soil in different villages (mg/kg, $\overline{\chi}\pm s$)

Group	Food (n=35)	Vegetable (n=51)	water-soluble fluoride in soil (n=9)		total fluorine in soil (n=9)	
			Plough layer	plow pan layer	plough layer	Plow pan layer
EFV+DFPV	1.77±0.25	0.58±0.34	126.09±18.64	120.91±28.71	37.90±20.45	39.97±14.90
CV	1.89±0.21	0.66±0.92	100.03±18.64	109.65±35.71	34.64±9.08	37.90±20.45
t	1.552	0.455	-3.084	-0.742	-2.564	0.236
P	0.130	0.651	**0.007**	0.469	**0.021**	0.817

3.3 The comparison of urine fluoride level, serum Ca^{2+} and Mg^{2+} level in different groups. (Table 3)

Fluoride levels in urine in different groups were: EFV>DFPV>CV, and there were significant differences in any two of the three groups (P<0.05 respectively). There were no significant differences of serum Ca^{2+} level among of the three groups (P>0.05). As for the serum Mg^{2+} level, there was significant statistical difference between CV and DFPV (P<0.05)

Table 3. The comparison of the concentrations of urine fluoride and serum Ca^{2+} and Mg^{2+} (mg/L, $\overline{\chi}\pm s$) of adults of EFV, DFPV and CV

Group	n	Urine fluoride*	Blood Ca^{+}	Blood $Mg^{+\#}$
EFV	157	2.50±1.50	89.62±13.74	22.51±4.18
DFPV	245	1.42±0.97	90.07±17.00	21.81±7.43
CV	492	0.98±0.50	87.84±20.56	23.31±5.84
F		173.605	1.383	5.090
P		0.000	0.251	0.006

* There was significant difference between any two of the three groups.

There was significant difference between DFPV and CV.

3.4 Correlation analysis between fluoride and Ca^{2+}, Mg^{2+} concentrations both in drinking water and in peripheral blood. (Table 4, 5)

Correlation analysis between fluoride and Ca^{2+}, Mg^{2+} concentrations in drinking water was shown in table 4, from which we can see that there was negative correlation between fluoride and Ca^{2+} concentration in drinking water of EFV（r=-0.509，P=0.044）; fluoride concentration was negative correlated with Mg^{2+} concentration in drinking water in CV（r=-0.720， P=0.005）.

Correlation analysis between the urine fluoride and the concentrations of Ca^{2+}, Mg^{2+} in peripheral blood of adults in different investigated villages was shown in table 5, the result showed that there was negative correlation between urine fluoride and blood Ca^{2+} in EFV (r=-0.183，P=0.022).

Table 4. Correlation analysis of concentrations of fluoride and Ca^{2+}, Mg^{2+} in drinking water (mg/L)

Group	n	Mg^{+}		Ca^{+}	
		r	*P* value	r	*P* value
EFV	157	-0.442	0.087	-0.509	**0.044**
DFPV	245	0.168	0.691	0.347	0.400
CV	492	-0.720	**0.005**	-0.190	0.535

Table 5. Correlation analysis between urine fluoride concentrations and the blood Ca^{2+} and Mg^{2+} concentrations of adults in EFV, DFPV and CV (mg/L)

Group	n	Mg^{+}		Ca^{+}	
		r	*P* value	r	*P* value
EFV	157	0.058	0.469	-0.183	**0.022**
DFPV	245	-0.031	0.626	-0.012	0.850
CV	492	0.020	0.664	0.086	0.058

4 Discussions

Endemic fluorosis is a major public health concern in China due to the excessive consumption of fluoride in drinking water, and this issue is not limited to China. India, one of the two most populous country of the world, is also the worst affected by fluoride. India is plagued with numerous water quality problems due to prolific contaminants mainly of geogenic origin and fluoride stands first among them. The weathering of primary rocks and leaching of fluoride-containing minerals in soils yield fluoride rich groundwater in India which is generally associated with low calcium content and high bicarbonate ions (Ayoob and Gupta , 2006).

Endemic fuorosis was caused by choronic persistent fluoride exposure due to the excessive consumption of fluoride through drinking water, brick tea, and coal-burning. In view of the influence of tea and coal consumption on chronic fluorosis, the fluoride levels in indoor and outdoor air, vegetables and crops were determined in the six villages. We found that most of the families cooked meals using wheat straw and cornstalk as the sources of energy both in endemic fluorosis villages and control villages because of their relatively undeveloped. The fluoride levels in indoor and outdoor air were all lower than the national standard and also no significant differences were found in different villages. Fluoride levels in grain, vegetables and plow pan layer of soils also had no significant differences in all six villages, which showed that with the exposure level, there were no accumulations in vegetables and crops and so on. But the water-soluble fluorine and total fluorine in plough layer were higher in endemic fluorosis villages than those in control villages. The results above showed that consumption of fluoride in drinking water is the major exposure pathway in the investigated villages. The high fluoride in plough layer maybe related with irrigation water with high fluoride level because the improved water was just used for drinking and cooking. Urine fluoride level represents the internal burden of individual exposed to fluoride. In current study, the fluoride concentrations in urine were higher in adults both from EFV and DFPV than those from CV. The fluoride level has been maintained at relatively higher level in local residents from DFPV even if the defluoridation project has been implemented for two years. It suggested that the excretion of accumulated fluoride from body is a long process. As we know, about half of the absorbed fluoride is quickly incorporated into developing bone and teeth, where nearly all of the body's fluoride is found (Padula and Macmillan, 2005). The absorbed fluoride by the skeleton is most efficient in children and decreases with age(Maudsley et al, 2004), but this process can continue up to age 55 (Merviel et al, 2005).

Major influences in fluoride metabolism, however, result from the simultaneous ingestion of fluoride and inorganic ions, e.g. calcium, magnesium and aluminum. It's well known that these ions, when present at higher levels in diet or water, will decrease fluorine absorption (Büttner, 1963). Previous study showed that fluoride can interfere in metabolism of trace elements, which maybe caused by the moderate to severe level of water fluoride concentration (Bian, 1993). In current study, there were no statistical significances of serum Ca^{2+} level among three groups; but serum Mg^{2+} level was lower in adults from DFPV than that from CV. On the other hand, urine fluoride was negative correlated with blood Ca^{2+} in EFV($r=-0.183$, $P=0.022$). It suggests that fluoride can affect on calcium metabolism and may combine blood Ca^{2+} to form indissolvable CaF_2 which can explain why individuals with high urine fluoride concentration have lower blood Ca^{2+} concentration.

Conclusion

In this study, it was found that the fluoride level has been maintained at relatively higher level in local residents even if the defluoridation project has been implemented for two years. It suggested that the excretion of accumulated fluoride from body is a long process. Individuals who have higher urine fluoride concentration tend to have lower blood Ca^{2+} concentration in EFV. It may suggest that fluoride can interfere in metabolism of calcium.

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