Original article

Serum calciotropic hormone levels, and dental fluorisis in children exposed to different concentrations of fluoride and iodine in drinking water

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Keywords: *fluoride; iodine; calcitonin; osteocalcin; fluorosis*

Background High fluoride exposure can result in dental fluorosis. Fluoride and iodine are coexistent in the drinking water of areas in China and may affect the prevalence of dental fluorosis and osteogenesis. The aim of this study was to investigate the relationship between serum calciotropic hormone level, and dental fluorisis in children exposed to different concentrations of fluoride and iodine in drinking water.

Methods A pilot study was conducted in three villages located in the Kaifeng and Tongxu counties of Henan Province, China in 2006. Children aged 8 to 12 years, born and raised in the three villages were recruited. The fluoride levels in the samples of urine from these children were detected by fluoride ion selective electrode. Calcitonin and osteocalcin levels in the serum, and serum calcium were measured by radioimmunassay and flame atomic absorption spectrometry, respectively.

Results Fluoride levels in urine were significantly lower in children from control area (CA) as compared with those from the high fluoride & iodine areas (HFIA) and the high fluoride area (HFA) (P <0.05 respectively), and no statistically significant difference was found between the children from HFIA and HFA. Additionally, calcitonin levels in the serum were significantly lower in children from CA and HFA as compared with that from HFIA (P <0.05 respectively), and osteocalcin levels in the serum was lower in children from CA than those from HFIA (P <0.05). No statistically significant difference in serum osteocalcin concentrations was found between children from HFA and HFIA.

Conclusion This study provides an evidence that iodine exposure may modify the serum calciotropic hormone levels related to fluorine exposure.

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Endemic disease is a type of systemic damage caused by severe mineral imbalances such as iodine deficiency disorder (IDD), and fluorosis, which is caused by excess intake of fluoride. In the past decades, many researchers focused on the health problems both in human and animals caused by a single chemical imbalance of environment, especially fluoride, iodine or arsenic. Previous studies have shown that high fluoride exposure can result in debilitating bone deformities, an increased risk of fracture, and many other problems resulting from skeletal fluorosis.[1,2] They have also shown that the degree of fluorosis in a population is directly related to the concentration of fluoride in drinking water.[3] It has been estimated that nearly 30% of the world's population is at risk for some form of IDD.[4] Insufficient intake of iodine is the most common cause worldwide of mental retardation and brain damage among children.[5-7] Goiter was also detected in local people in some areas with high iodine concentrated drinking water.[8-11] In recent years, researchers in China have also paid more attention to health problems caused by joined effects of several chemicals such as iodine and fluoride, fluoride and arsenic, and so on.[10] This is because several chemical elements are coexistent in many areas in China, such as iodine deficiency & fluoride excess or both excess in drinking water. It is possible that multiple chemicals in the same environment (i.e., in the drinking water) may have an antagonistic or a synergistic effect on human health. Zhao et al[12] reported that iodine deficiency increased the incisor fluorosis incidence and severity of the injuries caused by excessive fluorine. The role of iodine influencing fluorosis induced by fluorine has been unclear. It appears reasonable to study the effects of iodine and fluorine on dental fluorosis and osteogenesis under various combinations of iodine and fluorine intake.

Calciotropic hormones, such as calcitonin and osteocalcin,

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are often employed as biomarkers of bone formation status and may also play a role in dental fluorosis. They are believed to be involved in the mineralization of matrix due to the Gla-dependent binding of calcium and the subsequent absorption of hydroxyapatite. Therefore, osteocalcin and calcitonin might correlate with fluorosis and serum calcium. Previous research found that mice deficient in osteocalcin due to targeted gene deletion developed a phenotype with a higher bone mass.[13] Thus, osteocalcin may be a negative regulator of bone formation. Our previous study suggested that the levels of serum osteocalcin in the dental fluorosis cases from high fluoride areas were higher than that from control area.[14] However, it is unclear if dental fluorosis prevalence or the level of serum calciotropic hormone can be affected when the fluorine and iodine co-existence in drinking water. We subsequently hypothesized that the iodine would modify the effect of fluorine on dental fluorosis and osteogenesis. Although previous studies have reported differences of iodide intake could increase/decrease the toxic effects of fluoride excess on the incisors and bones,[12] very few studies focus on the effect of different concentrations of fluoride & iodine on dental fluorosis and osteogenesis. We conducted a pilot study in Henan Province, China to investigate the serum calciotropic hormone levels, and dental fluorisis in children exposed to different concentrations of fluoride and iodine in drinking water.

METHODS

Location and population

A pilot study was conducted in three villages located in the Kaifeng and Tongxu counties of Henan Province, China in 2006. They were divided into three areas according to different concentrations of fluoride and iodine in drinking water : 1) high fluoride and iodine area (HFIA), the average concentration of fluoride and iodine in drinking water were 3.1 mg/L and 397.5 μg/L respectively; 2) high fluoride and normal iodine area (HFA), the average concentration of fluoride and iodine in drinking water were 1.8 mg/L and 91.8 μg/L respectively; 3) control area (CA), the average concentration of iodine in drinking water was 76.3 μg/L and the average concentration of fluoride in drinking water was <1.0 mg/L. There were no significant differences in the natural environment, socioeconomic status, life styles, dietary habit, and other demographic characteristics among these three areas. All the three areas have similar levels of calcium and magnesium in drinking water and food.

Children aged 8 to 12 years old, born and raised in these three areas were recruited excluding children who have received drug treatment in the form of bisphosphonates, calcitonin, fluoride, or hormone replacement therapy and/or who had hip fractures. A total of 198 children participated in this study, 60 from HFIA, 66 from HFA, and 72 from CA. All participants were examined for dental fluorosis using Dean's Method.[15] Children who were diagnosed as grade 0 or 1 were classified as non-dental fluorosis, whereas those who were diagnosed as grade 2, 3, 4, or 5 were classified as dental fluorosis. Dental fluorosis prevalence (DF%) and community fluorosis index (CFI) were used as statistical indicator. DF% = (the number of who were diagnosed as grade 2, 3, 4, and 5 / the total number of checked) × 100%, and CFI = ((the number of questionable × 0.5) + (the number of very mild × 1) + (the number of mild × 2) + (the number of moderate × 3) + (the number of severe × 4))/the total number of checked. Each child provided about 6 ml of fasting blood and 10 ml of instant urine samples. All procedures were approved by the Institutional Review Board at Zhengzhou University, China (IRB 00006861, FWA00014064).

Detection of urine fluoride

The fluoride levels in the samples of urine from the three areas were detected by fluoride ion selective electrode (Shanghai Exactitude Instrument Company, China) during the study. The high-loaded fluoride status was classified if the urine fluoride concentration exceeded 1.5 mg/L.

Calciotropic hormones measurements

Serum was stored in a –20°C freezer and was not thawed prior to assay for calcitonin (CT, Center of Science and Technology Explore, General Hospital of PLA, Beijing, China) and osteocalcin (OC, Chemclin Biotech, Beijing, China) levels using commercial radioimmunoassays. The intra- and inter-assay variation was <10% for these assays. Otherwise, serum calcium level was measured by flame atomic absorption spectrometry (Hitachi Z-5000, Japan) with recoveries in the range of 98.0%–110.2%.

Data analysis

Differences in age and gender distribution as well as high-loaded fluoride status were analyzed using the chi-square test. Differences of fluoride in urine, calcitonin and osteocalcin in serum among different groups were examined by analysis of variance (ANOVA). All significant tests were two-sided. A *P* value of less than 0.05 was considered statistically significant. All analyses were performed using the SPSS Software, version 12.0 (SPSS In., Chicago, USA).

RESULTS

There were 67 subjects who were diagnosed with dental fluorosis. All of them lived in HFIA or HFA (Table 1), the prevalence rates of dental fluorosis were 53.3% (32/60) and 53.0% (35/66) in children of HFIA and HFA respectively, no dental fluorosis cases were found in CA. The community fluorosis indexes were 1.56, 1.24, 0.03 in children of HFIA, HFA and CA respectively. The prevalence of high-loaded fluoride status in HFIA was 93.3% (56/60), while the prevalences in HFA and CA were found to be 66.7% (44/66) and 11.1% (8/72), respectively. The prevalences of high-loaded fluoride

Table 1. Distributions of select variables in different areas

Variables	HFIA (*n*=60)	HFA (*n*=66)	CA (*n*=72)
Age* (years)	9.90±1.34	9.45±1.24	9.70±1.00
P values		0.04†	0.65
Gender (*n*)			
Male	26	36	36
Female	34	30	36
High-loaded fluoride status (*n*)			
Yes	56	44	8
No	4	22	64
P values		0.08	<0.01
Dean's Scoring (*n*)			
0	25	22	68
1	3	9	4
2	0	11	0
3	13	11	0
4	10	8	0
5	9	5	0
Dental fluorosis			
Rate (%)	53.33	53.03	0
P value			0.00
CFI	1.56	1.24	0.03

*: values were shown as means ± SD. †: HFA compared with HFIA.

status in HFIA and HFA were higher than that in CA (*P* <0.01), and no differences between the two groups were found. The age distribution of children from HFA was lower than those from HFIA, and no differences were found between children from CA and HFIA, as well as CA and HFA. The gender distribution among children from HFIA did not significantly differ from that of HFA and CA.

We compared levels of urine fluoride, serum calcium, serum calcitonin and osteocalcin among all the children from these three areas (Tables 2 and 3). Compared with children from HFIA and HFA, the level of urine fluoride was significantly lower in children from CA (*P* <0.05 respectively). However, no statistical significance was found between children from HFIA and HFA (*P* >0.05). Statistically significant differences in serum calcium levels were not found among children from the three areas (*P* >0.05).

Table 2. Urine fluoride and serum calcium measurements in different areas

Groups	*n*	Fluoride* (mg/L)	Calcium* (mmol/L)
HFIA	60	2.70±0.32†	2.59±0.46
HFA	66	2.04±0.89†	2.40±0.47
CA	72	0.84±0.49	2.49±0.52

*: data were shown as means ± standard error (SD). †*P* <0.05 vs. CA group.

The levels of serum calcitonin were significantly lower among children from CA and HFA compared to those from HFIA (*P* <0.05 respectively), and no significant difference was found between CA and HFA (Table 3). The level of serum osteocalcin was lower in children from CA compared with those from HFIA (*P* <0.05). Statistical significance in serum osteocalcin concentrations was not found between children from HFA and HFIA (Table 4).

Table 3. Serum calcitonin measurements in children from different areas

Groups	*n*	Serum calcitonin (ng/L)	*F* value	*P* value
HFIA	60	268.69±170.54		
HFA	66	172.35±87.14*	9.05	0.00
CA	72	137.37±72.19*		

**P* <0.05 vs. HFIA group.

Table 4. Serum osteocalcin measurements in children from different areas

Groups	*n*	Serum osteocalcin (ng/L)	*F* value	*P* value
HFIA	60	6.84±3.91*		
HFA	66	6.21±1.40	4.65	0.01
CA	72	5.39±1.29		

**P* <0.05 vs. CA group.

DISCUSSION

Endemic goiter and fluorosis are major public health concerns in Henan province due to the consumption of various concentrations of fluoride and iodine in drinking water. But to date, studies of fluorosis mainly focus on bone deformities and non-bone toxicity, including the change of some biochemical enzymes and hormones caused by fluoride only. The role of the combination of fluoride and iodine in drinking water in children serum calcium and calciotropic hormone levels has been unclear.

Previous study showed that dental fluorosis increased along with fluoride concentration in drinking water.[3] In this study, we did not find the difference in dental fluorosis rate between HFIA (53.3%) and HFA (53.03%) although the fluoride concentration in drinking water in HFIA (3.1 mg/L) was higher than that in HFA (1.8 mg/L). HFIA was the moderate-grade endemic fluorosis area and HFA was the low-grade endemic fluorosis area according to the national standard for the classification in endemic fluorosis areas (GB 17018-1997).[16] Higher iodine concentration in drinking water in the HFIA may influence the incidence of dental fluorosis. The results from Zhao et al[12] supported our findings. In their experiment in mice, the dental fluorosis prevalence of iodine & fluoride excess group was found to be significantly lower compared with groups of deficiency/normal iodine and excessive fluoride. They also found that iodine deficiency increased the incidence of incisor fluorosis and the severity of the injuries caused by excessive fluorine. However, we did not observe the difference in the severity of the dental fluorosis in the two high fluoride areas although the community fluorosis index in HFIA (1.56) was slightly higher than that in HFA (1.24). Urine fluoride reflects the fluoride level in the body. Higher fluoride levels in urine among children from HFIA and HFA showed that high-loaded fluoride status of the body in the children from the two high fluoride areas. It suggested that higher iodine level in drinking water did not change the fluoride concentration in urine. The result from Yang et al[17] also showed that children in the high fluoride and high iodine areas had higher fluoride level in urine compared with those in the control areas.

Osteocalcin (BGP-Bone Gla Protein) is an important biochemical factor associated with bone metabolism. It is

synthesized exclusively by osteoblasts and is well-known as a marker for differentiated mature osteoblasts and is an important determinant of the bone mineralization process.[18,19] Although osteocalcin has been suggested to be involved in dental or skeletal de- and remineralizing processes, its relationship with bone development is unclear currently.[13,20-22] On the other hand, fluoride incorporated into bone during mineralization.[23] Studies have shown that skeletal fluorosis patients had a greater number of osteoblasts than controls.[24] Farley et al[25] and Kebsch et al[26] believed that fluoride influences bone growth by acting as a mitogenic agent for osteoblasts and might also act as an inhibitory stimulus to osteoclasts, although this is not well understood because of the possible side effect of fluoride-induced secondary hyperparathyroidism. In our study, the osteocalcin levels in serum increased sequentially with the increasing fluoride concentration in drinking water. The high serum levels of osteocalcin among children with high fluoride & iodine exposure observed in the current study may reflect that the high number and/or high activity of osteoblasts in those children. Huang et al[27] found that serum osteocalcin concentration were significantly increased in both male and female rats in the high-iodine intake experimental groups compared with the low-iodine intake group after 6 months of different iodine concentration intake. Ohta et al[28] found that serum osteocalcin levels were significantly increased in fluoride-treated rats compared with the controls. Hence, it is possible that as a mitogenic agent, fluoride may affect bone formation by regulating the concentration of osteocalcin in serum. Iodine may change the level of thyroxine and then further to affect on the secretion of osteocalcin. Although the serum levels of osteocalcin were increased in children exposed to high fluoride only, this increase was not statistically significant compared to CA. It suggested that it did not bring the significant effect on the osteocalcin level in the serum when the fluoride concentration in drinking water was less than 2.0 mg/L. Kebsch et al[26] found that fluoride can increase the mean of osteocalcin concentration, but it was not found to be statistically significant compared to the control group.

Calcitonin, discovered more than 40 years ago, is a 32-amino-acid polypeptide produced in humans by the parafollicular cells of the thyroid gland.[29] It plays an important role in regulating bone formation and affecting osteoblasts although as yet has an incompletely defined role in calcium homeostasis. *In vitro* and *in vivo* studies showed that calcitonin was effective in inhibiting osteoclast activity, thereby reducing bone resorption.[30] Wallach et al[31] reported in an animal model that calcitonin increased bone growth and biochemical markers of bone formation. In the present study, the serum calcitonin levels among children from HFIA were higher than that of HFA and CA. No difference was found between children from HFA and CA. It suggested that iodine can inhibit osteoclast activity by increasing the serum concentration of calcitonin and then reducing bone resorption caused by high fluoride. Rao[32] believed that calcitonin would be decreased and the relationship between thyroxine and some other calcium related hormones such as calcitonin would be imbalance when blood thyroxine decreased. Iodine may improve the calcitonin concentration by regulating the concentration of blood thyroxine. On the other hand, the level of calcitonin is closely related to the level of calcium. The higher levels of calcium, the higher level of calcitonin.[33] In this study, serum calcium levels of these three areas have no statistical significance, but it was slightly higher in HFIA compared with other two areas. The results above indicated that iodine may modify the effect of fluoride on serum calciotropic hormone levels among children. In conclusion, this study provides evidence that iodine exposure may modify the serum calciotropic hormone levels related to fluorine exposure. This suggests that a new biomarker of dental fluorosis may be used to identify high-risk populations in areas with high levels of fluoride in drinking water. The regulation of calciotropic hormones is a complex system, so future studies are needed to confirm this finding in a large population and additional related hormones.

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