

SERUM OSTEOCALCIN AND CALCITONIN IN ADULT MALES WITH DIFFERENT FLUORIDE EXPOSURES

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SUMMARY: The concentrations of serum osteocalcin (OCN) and calcitonin (CTN) were determined in sixty male workers exposed to fluoride (F) at an aluminum plant in Danjiang city, and in thirty non-F exposed males of the same general age from the local market town Gaolou village of Jun county in Danjiang city (control group). The F-exposed workers were divided into two groups according to the levels of their urine and serum F: a high-F burden group (urine F>4.0 mg/L; serum F>0.20 mg/L) and a low-F burden group (2.0 mg/L<urine F≤4.0 mg/L; 0.10 mg/L<serum F≤0.20 mg/L). Compared with the control group, the concentrations of serum OCN and CTN were significantly higher in both the high-F and low-F burden groups (p<0.05). This study found for the first time that the concentrations of serum OCN and CTN increased concurrently in a F-exposed worker population. On the basis of these findings, we propose that serum OCN and CTN might be sensitive biomarkers for detecting early stages of F bone injuries.

Keywords: Aluminum plant workers; Calcitonin (CTN); Fluoride bone injuries; Osteocalcin (OCN); Serum fluoride; Urine fluoride.

INTRODUCTION

The main hard tissue injuries from fluoride (F) are dental fluorosis and bone sclerosis. In the latter, earlier activation of osteoblasts (OBs) apparently plays a leading role. In their studies, Krook and Minor found that F had negative effects on bone cell metabolism, resulting in pathological bone formation and reducing bone resorption,¹ which may be the main cause of bone sclerosis. With excessive exposure to F, *OBs appear to be a primary focus of bone injury in the pathogenesis of chronic fluorosis.*² Recent research has indicated that F impacts the adult skeletal system mainly through calmodulin and the activity of OBs. As is known, osteocalcin (OCN) is mainly synthesized and secreted by OBs, whose activity is reflected by OCN specifically. In addition, calcitonin (CTN) is an important calcium regulating hormones, so the combined role of OCN and CTN in bone metabolism has attracted increasing attention.³

In this study, as a possible aid to detect high-risk individuals for developing skeletal fluorosis, serum OCN and CTN in F-burden adult males were determined to detect early changes and mechanisms of F bone injuries.

MATERIALS AND METHODS

Subjects and sampling: The subjects of this study were sixty F-exposed male workers, 18 to 50 years of age, from an aluminum plant in Danjiang, a city of Central China's Hubei Province, and thirty non-F exposed males of the same age range from the local market town, the Gaolou village of Jun county in Danjiang city, located about 3 km northeast and in the direction of the prevailing winds from the aluminum plant. Anyone who had consumed hormones during the preceding 3

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months, suffered from liver and/or kidney disease, or other illness that might affect the absorption and excretion of F, was excluded. The sixty F-exposed workers were divided into two groups of 30 each according to the concentrations of their serum F and urine F: the high-F burden group (urine F>4.0 mg/L and serum F>0.20 mg/L) and the low-F burden group (2.0 mg/L<urine F≤4.0mg/L; 0.10mg/L<serum F≤0.20mg/L). The thirty controls had lower urine and serum F levels. All the samples of blood and urine were collected with informed consent.

Methods of determination: The concentrations of serum F and urine F were determined by the electrode method and trace F-ion selective electrode and the concentrations of serum OCN and CTN were measured by competitive radioimmunoassay, with kits of Serum OCN and CTN provided by the Institute of Atomic Energy Atomic Hi-Tech Co. Ltd of China.

Statistical analysis: All data are presented as mean values with standard deviations (SD). Statistical tests for significance were performed using the Student *t* test by SPSS12.0. Differences with $p<0.05$ were considered to be statistically significant.

RESULTS

As shown in the table, the concentrations of both serum OCN and CTN in the high-F and low-F exposed burden groups were significantly higher than those in the control group ($p<0.05$).

Table. Concentrations of serum OCN and CTN in each group (values are mean ± SD)

Group	Number of cases	Osteocalcin (ng/mL)	Calcitonin (pg/mL)
Control	30	4.87±1.85	59.96±11.16
Low-dose F	30	5.99±2.03*	71.92±13.29*
High-dose F	30	7.37±2.18*	84.11±15.45*

*Compared with the control: $p<0.05$.

DISCUSSION

Related studies have shown that the main hazard of F includes bone sclerosis (96%) and calcification of ligaments and pseudofracture (50%),⁴ which are mostly caused by disorders of OB and osteoclast (OC) metabolism.¹ OCN is a bone protein synthesized by OB, and about one-third of it is directly distributed into blood circulation. It plays a very important role in maintaining normal mineralization of bone, inhibiting calcification of cartilage and deposition of irregular crystals.⁵⁻⁷ Consequently, serum OCN increases when bone is synthesized, so its levels and degrees of carboxylation reflect the specific activity of OBs, the level of OCN in bone tissue, and the condition of F bone injuries. Our results show that the concentration of serum OCN became greater with increasing F load, and there were significant differences in both the high-F and low-F group ($p<0.05$) compared with the control group, which could reflect an increased activity of OB in the two F groups.

Some research has shown F can promote OB proliferating *in vitro* at a certain dose,⁸ possibly by the following mechanisms: (1) direct effect of F on OBs; (2) changes in the regulation of oncogene expression that could ultimately promote proliferation of OB; and (3) stimulation of signal transduction pathway of OB proliferation. These mechanisms are likely to be primary causes, which lead to higher levels of serum OCN in F overload, thereby increasing bone injury by F, but the exact regulatory mechanisms are still unclear and require further study.

CTN (calcitonin) is a polypeptide hormone secreted by the parathyroid gland that is involved in bone metabolism by regulating the balance of calcium, and inhibiting the activity of OCs while enhancing the activity of OBs by stimulating their formation.⁹ *In vitro* tests indicate that F can directly activate OBs and improve the expression of metalloproteinases-9, which could boost the activity of OC bone resorption.^{10,11} However, our findings showed that the level of serum CTN in the F-load groups was higher than that in the control group, a finding supported by Okayasu et al., whose work also showed that serum values of CTN in F groups were higher in comparison with controls.¹² In research by Teotia et al. the CTN levels in seven patients with endemic skeletal fluorosis were higher than those in normal subjects.¹³ Similarly, Krishnamachari and Sivakumar observed a significant elevation of CTN in fifteen subjects afflicted with skeletal fluorosis with or without *genu valgum*.¹⁴

In contrast to these findings, Gao et al. found that there were no differences in CTN between a F load and a control group in a dynamic analysis of effects of subchronic fluorosis on bone turnover in rats.¹⁵ Ma and her co-workers also found that serum CTN values in a high-F area were lower than those in the control areas.¹⁶ Krook and Minor reckoned that the high dietary calcium eventually caused hypocalcemia and osteopetrosis, because of CTN production's increasing.¹ Furthermore, we noticed that, in these reports, there was an obvious relationship between serum CTN and calcium ion (Ca^{2+}): the serum CTN level rose when the Ca^{2+} level was normal, and the former decreased when the latter declined. This fact indicates that the levels of serum CTN in fluorosis are directly regulated by the level of serum Ca^{2+} , which is negatively affected by the serum PTH. Therefore, serum Ca^{2+} appears to be closely related to the disorder of hormone regulation in skeletal fluorosis. The two contrary results in the above-mentioned reports might be mainly to the differences of serum Ca^{2+} between two groups.^{15,16} We speculate therefore that a high Ca^{2+} diet may reduce the morbidity of F injury to bone.

Our results demonstrated that long-term intake of excessive F caused a concurrent increase in serum OCN and CTN, which might contribute to further manifestations of F-bone injury in which active bone transformation occurs and osteogenesis plays a major role. With an increase in the concentration of serum CTN, bone metabolism is changed, and the normal balance between OB and OC activity is disrupted. According to our results, serum OCN and CTN might therefore be early sensitive biomarkers of F bone injuries. But the fundamental regulatory mechanisms—how the serum CTN accommodates the balance of

calcium, attenuates the activity of OCs while boosting the activity of OBs in the state of F load, and whether F directly activates OBs and leads to metabolic bone disturbance, etc.—are still not entirely clear and need further in-depth study.

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