EFFECTS OF FLUORIDE ON GROWTH PLATE CARTILAGE IN RATS: RADIOLOGICAL AND HISTOPATHOLOGICAL FINDINGS

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SUMMARY: The aim of this study was to investigate the effects of ordinary and high doses of fluoride on rat growth plate cartilage and surrounding bone. Thirty-six newborn Wistar albino male rats were divided equally into three groups and, three weeks after birth, were given the same 1.2 ppm (control group), 100 ppm, and 150 ppm fluoridated water their mothers had been drinking. Radiological and histopathological examinations were performed in the 15th, 17th, 20th, and 23rd weeks. There were significant histopathological variations in chondrocyte morphology and extracellular matrix volume in the zone of growth plate between the control group and the fluoride-treated groups. The main histopathological findings in the rats treated with high doses of fluoride were a decrease in matrix volume and cartilage septae thickness, and an increase in the number and diameter of chondrocytes on growth plate. On the epiphyseal ossification center, irregular lamellae, minimally increased osteoblast numbers, rare zones of woven bone, and irregular and thinned trabeculae were observed. On radiological examination, the main findings were relative widening and late partial fusion of the growth plate of high-dose fluoride treated groups.

Keywords: Bone growth; Bone histopathology; Cartilage; Fluoride and bone; Growth plate; Histopathology; Radiology.

INTRODUCTION

Fluoride is a cumulative toxin which can affect bone tissue and mineralization processes.[1] In the majority of affected communities, fluoride in drinking water is the primary source of dental fluorosis and skeletal fluorosis,[2] which has various histopathological and radiological appearances. These changes range from diffuse osteosclerosis to diffuse osteopenia and may resemble features of hyperparathyroidism, rickets and osteomalacia.[3]

Growth plate (GP) is a highly specialized cartilage structure that is responsible for the longitudinal growth of bone. GP cartilage is divided histologically into three zones by name: reserve zone, proliferative zone, and hypertrophic zone. During growth, there is a dynamic transformation of GP cartilage into new trabecular bone; this is achieved by calcification of the cartilage extracellular matrix in lower zone of GP. The calcified cartilage matrix produced in the GP provides the template for the production of primary spongiosa in the metaphyseal region. Longitudinal bone growth continues until fusion of growth plates occurs.[4,5] Factors affecting development and maturation of GP cartilage and bone elements may eventually lead to disturbances of growth. Mineralization defects that are

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accepted as hallmarks in the pathological diagnosis of skeletal fluorosis are also a part of the picture of bone histopathology seen in the childhood skeletal disorder known as rickets.[6]

Bone fluorosis has been studied extensively by both radiological and histopathological methods. However, there is limited information about the effect of fluoride on GP. In this study, we investigated the effects of high doses of fluoride on rat GP and surrounding bone during growth.

MATERIALS AND METHODS

Study Design: Thirty-six newborn male Wistar albino rats were used for the study and were divided into three groups of 12. The rats were maintained under a 12-hr light:12-hr dark cycle in a temperature controlled room (22-24 ºC) with six rats housed per cage. The animals were fed a standard a rodent chow diet and given drinking water containing 1.2 ppm F^- in group I (control), 100 ppm F^- in group II, and 150 ppm F^- in group III after they were weaned three weeks after birth. Fluoridated drinking water was prepared by dissolving sodium fluoride in deionized water and analyzing for fluoride ion content before use. Since GP radiologically fuses at 17–21 weeks of age in rats,[7] the longest duration of fluoride exposure was planned to be 23 weeks. Radiologic examinations were performed in the 15th, 17th, 20th, and 23rd weeks. Three rats from each group were sacrificed by ether inhalation at these periods, and femora were used for histopathological evaluation.

Histopathology: Femur samples of rats were fixed in 10% formalin for 24 hr and decalcified in 7% formic acid. Longitudinal sections on the frontal plane containing the epiphysis, growth plate, metaphysis, and metaphyso-diaphyseal region on the distal femur were collected. All sections were embedded in paraffin. Five-micrometer thick sections were cut on rotary microtome (Leica RM2155, Germany) and were stained with hematoxylin and eosin (HE). The histopathological slices were examined with light microscopy (Nikon-optiphot-2, Japan) in a blind fashion.

Radiology: The radiographic examinations were performed at a mammography unit with a magnification technique (MD 4000, Philips, The Netherlands) to achieve good image quality. Radiographies were obtained in prone position to cover the pelvis and lower extremities. Lower epiphyseal growth plate of the femur and upper epiphyseal growth plate of tibia and fibula were carefully examined as well as the main radiographic feature of the surrounding bone to evaluate the degree of fusion of growth plates.

RESULTS

Histopathology: There were significant histopathological differences between the control group I and groups II and III given high doses of fluoride. More and larger chondrocytes and long columns were observed in proliferative and hypertrophic zones of groups II and III. The extracellular matrix volume and cartilage septae thickness in proliferative and hypertrophic zones were narrower in groups II and III than in group I (Figure 1). Moreover, an increase in the number of vessels and irregular and thinned trabeculae was seen in primary spongiosa of groups

II and III. Major histopathological findings of the epiphyseal ossification center of the rats in groups II and III included irregular interstitial lamella, rare zones of woven bone, and a minimal increase in osteoblast number which sometimes appeared in multiple layers on bone sections (Figure 2).

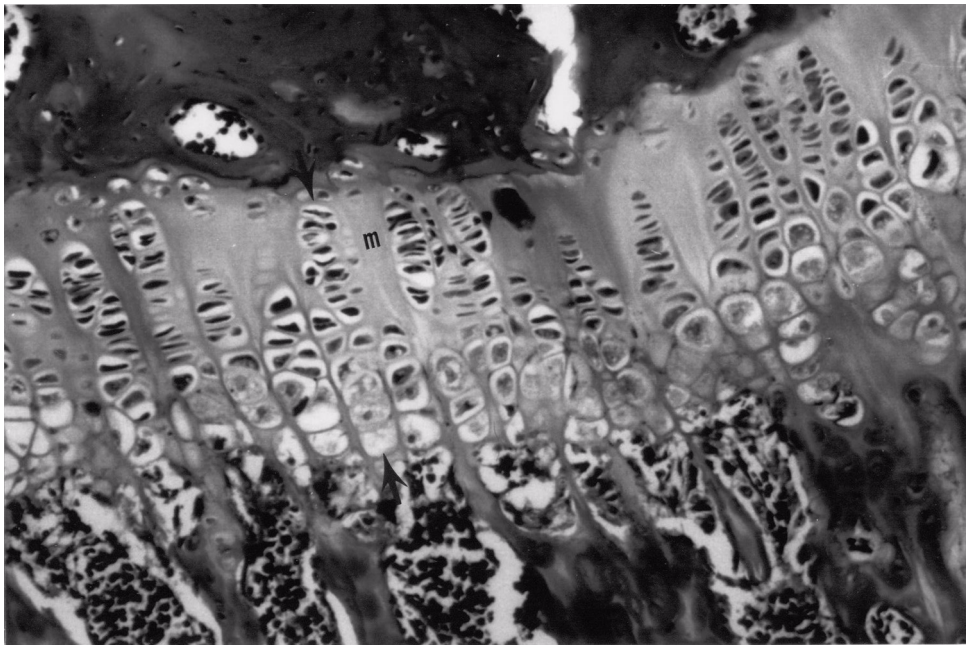

Figure 1a.

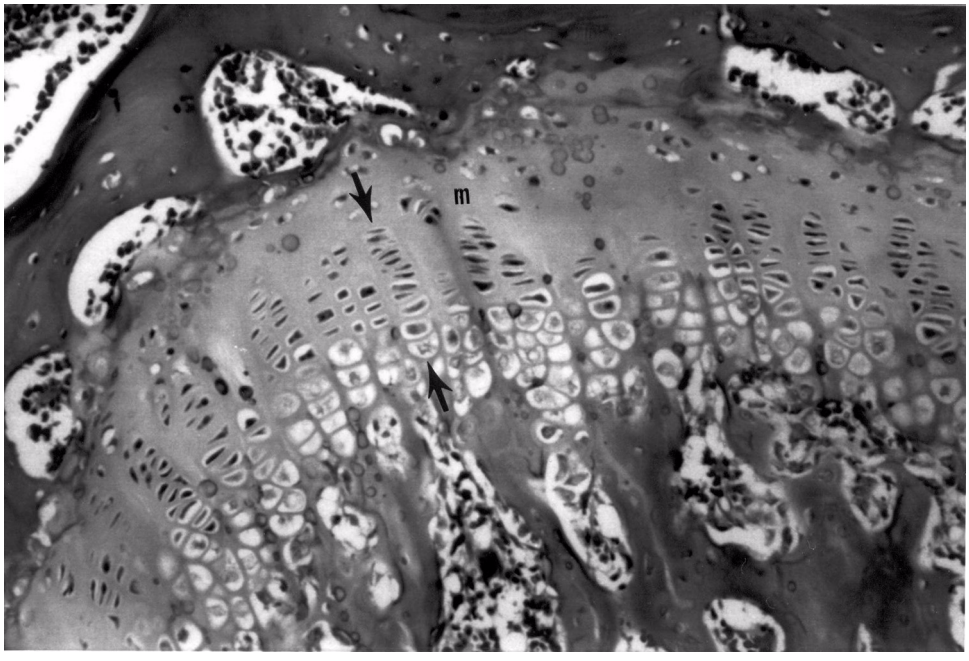

Figure 1b.

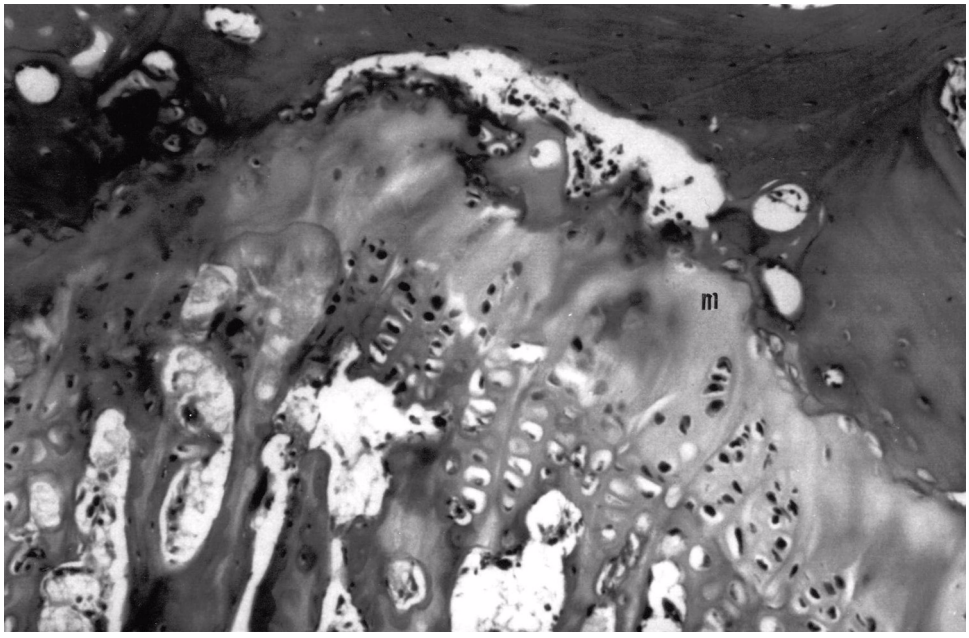

Figure 1c.

Figure 1. Histopathological section of rat distal femur GP (HE x 100), longitudinal plane, group III (150 ppm F) (a) and group II (100 ppm F) (b) at 23rd week; more and larger chondrocytes, forming high columns (between arrows), and relatively decreased extracellular matrix (m) volume are observed compared to group I (control) (c). Note that the changes are more noticeable in group III than in group II.

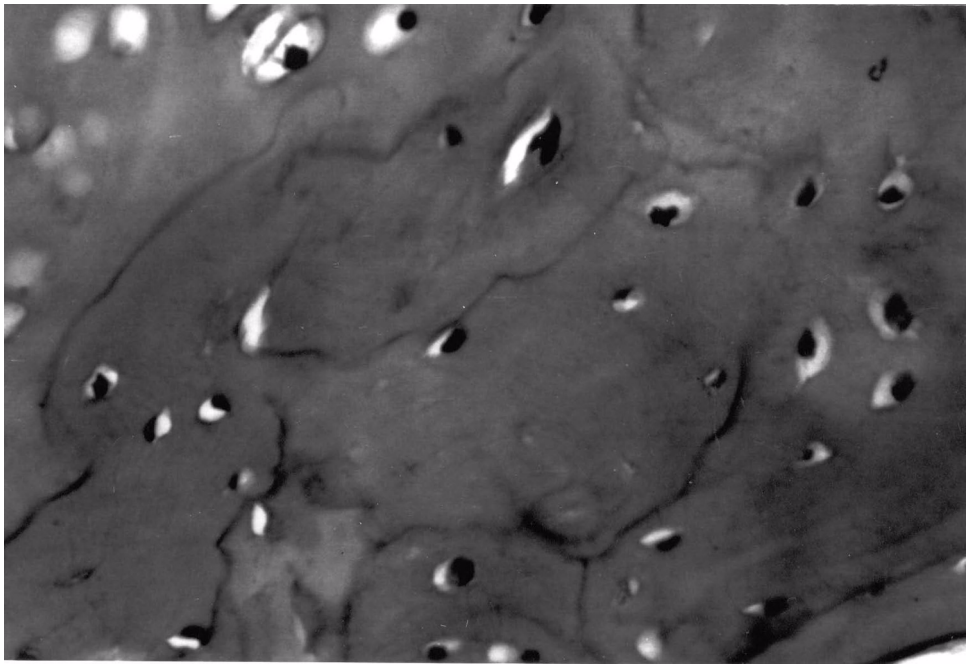

Figure 2a.

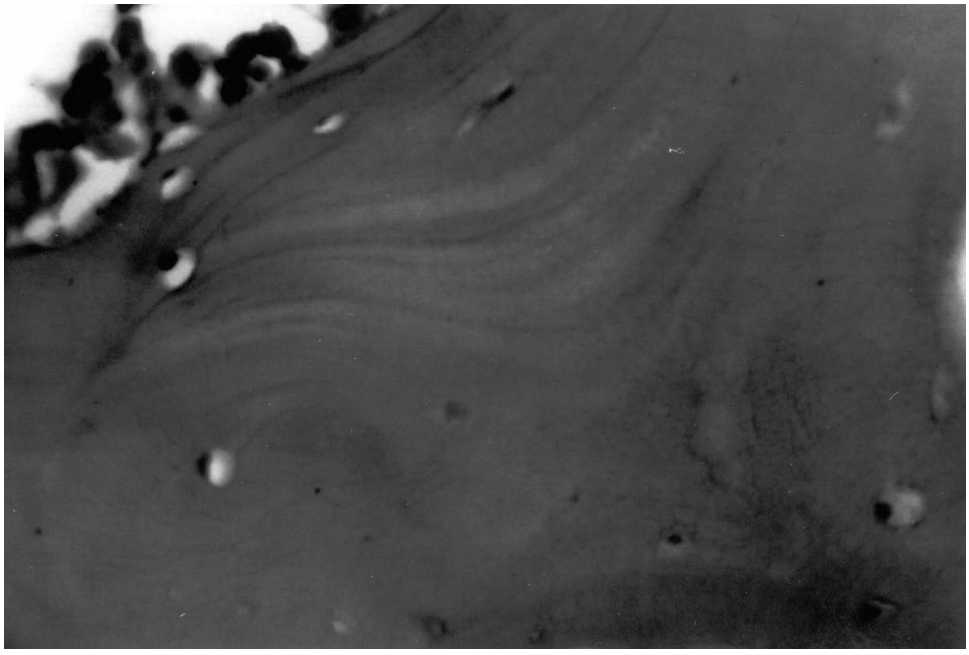

Figure 2b.

Figure 2. Histopathological section of rat epiphyseal ossification center (HE x 400), longitudinal plane, group III (a), at 23rd week; Irregular interstitial lamellae and woven bone and minimally increased osteoblasts are noted compared to group I (control) (b).

Radiology: The fusion of growth plates was found to be delayed in groups II and III compared to group I. In addition, the fusion of GPs of rats in group III was later than in group II. Discernible radiographical findings were observed on tibia and fibula GPs. The fusion of femur GPs was earlier than that of tibia and fibula GPs in all groups. At the 15th and 17th weeks, growth plates were open in all groups. At the 20th week, in group I, GPs of femora were completely fused, GPs of the tibia and fibula were partially fused. In groups II and III, none of GPs were completely fused. By the 23rd week (Figure 3), all growth plates were completely fused in group I. In group II, the GPs of femora were also completely fused, but GPs of tibia and fibula were only partially fused. In group III, none of the GPs were completely fused: GPs of femur were partially fused, but GPs of tibia and fibula were still open.

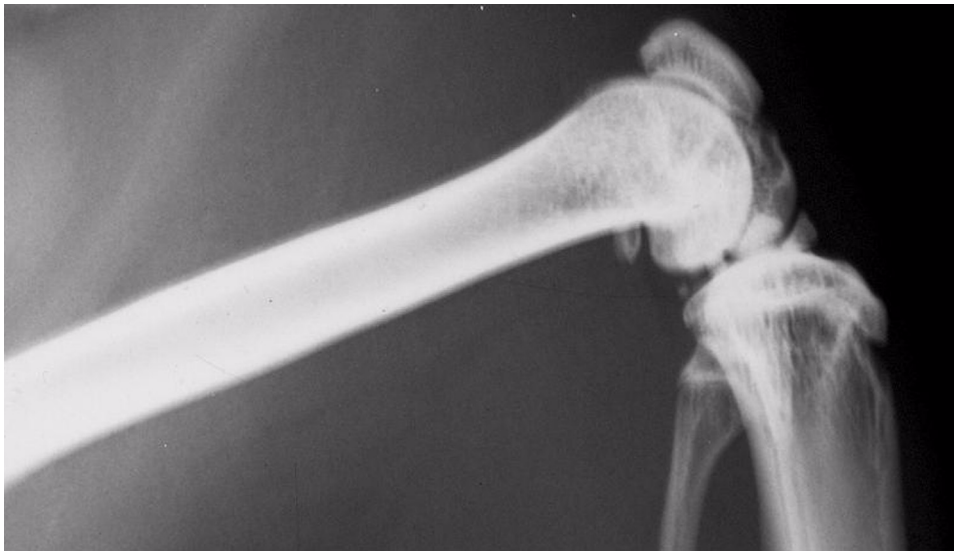

Figure 3a.

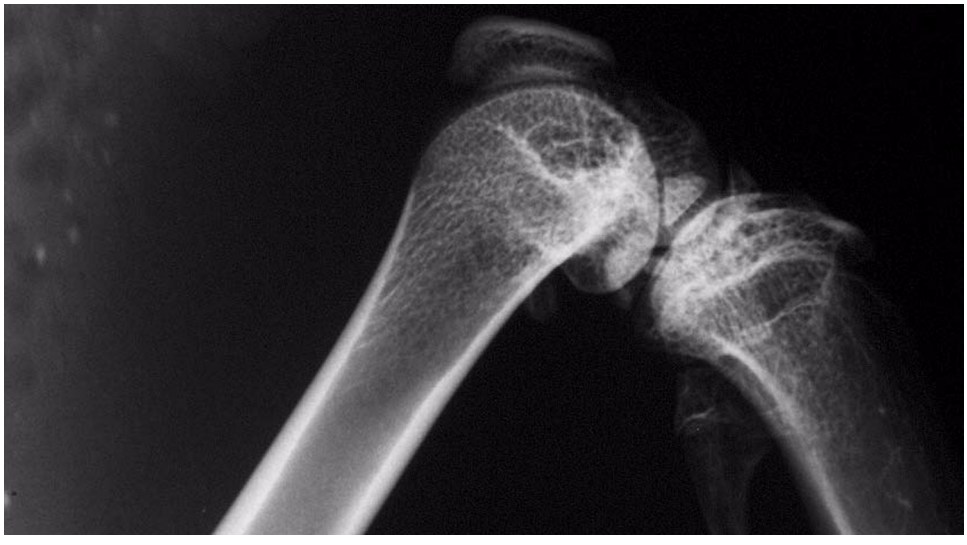

Figure 3b.

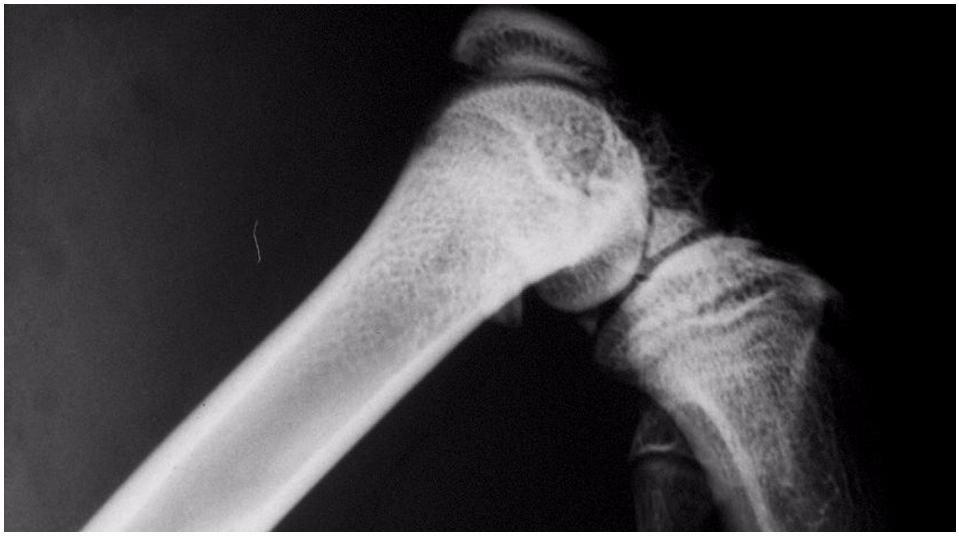

Figure 3c.

Figure 3. Radiographs of rats in groups I (a), II (b), and III (c) at 23rd week. Complete fusion of all GPs are observed in group I. Complete fusion of femur GP and partial fusion of tibial and fibular GPs are seen in group II. In group III, the femur GP is partially fused but tibial and fibular GPs are still open.

DISCUSSION

The main function of the growth plate is to facilitate longitudinal bone growth. This process is accomplished through cellular proliferation and maturation, matrix production and mineralization, and endochondral ossification and resorption. The function of the proliferative zone is matrix production and cellular division, which together contribute to longitudinal growth. The function of the hypertrophic zone is to prepare the matrix for calcification and to calcify it. The calcification of the cartilage extracellular matrix in the lower region of the hypertrophic zones plays a major role in its transformation into trabecular bone.[4,5,8]

Skeletal growth requires harmonized development and maturation of GP cartilage and bone elements. Within GP zones, an internal rearrangement of tissue structure occurs. The height of the proliferative and hypertrophic zones within the GP normally decreases with increasing age. The matrix volume fraction increases with age in each zone. Factors affecting these processes may eventually lead to disturbances of growth.[5] In this study, we investigated the effects of high doses of fluoride in drinking water on the GP and surrounding bony architecture of young rats radiologically and histopathologically. Our histopathological findings showed that high doses of fluoride affected the zones of GP. The extracellular matrix volume and cartilage septae thickness in the proliferative and hypertrophic zone of fluoride-treated groups were diminished, due to the enlargement of the cells. Besides, irregular and thinned trabeculae were seen in epiphyseal ossification centers and primary spongiosa in fluoride-treated groups. These

radiological findings thus showed that high levels of fluoride exposure lead to delay in the fusion of GPs. A parallel delay in the fusion of GP also occurred with an increase in fluoride exposure. Thus the amount of fluoride exposure is an important factor in modifying the fusion of growth plate during growth.

The histopathological changes in bone fluorosis are variable.[9] The main histological change induced by fluoride is the increase of the osteoid volume with trabecular thickening.[10] However, thinned and atrophied trabeculae, defective mineralization, and bone resorption can also be seen.[11,12] Fluoride induces cell injury in both osteoblasts and resorbing osteocytes. Krook and Minor[13] propose that the increased amount of trabecular bone results from pathological bone formation by injured osteoblasts and decreased bone resorption by resorbing osteocytes and osteoclasts. Boivin *et al*[14] reported only rare zones of woven bone, and they mainly noted an altered lamellar appearance in a histomorphometric study of skeletal fluorosis. We also found out irregular lamellar orientation, rare zones of woven bone, and a decrease in trabecular thickness on epiphyseal ossification center of rats treated with high levels of fluoride.

Recently, Guo *et al*[15] found that excessive fluoride ingestion (EFI) significantly alters the differential expression of collagen phenotypes and chondrocyte differentiation in the cartilage of rats. They found that abnormal collagen expression in rats of the EFI group resulted from abnormal chondrocyte differentiation in proliferative and hypertrophic zones in growth plate cartilage and rib cartilage and from degenerative changes in articular cartilage. From their results they propose that abnormal chondrocyte differentiation in hyaline cartilage of rats with skeletal suggests two pathogenic mechanisms for early stage skeletal fluorosis: (1) osteomalacia-like changes in growth plate cartilage with delayed mineralization and (2) delayed mineralization and degenerative changes in articular cartilage. These conclusions are concordant with our findings, since we also observed similar changes in our rats exposed to high levels of fluoride.

Skeletal fluorosis has various radiological appearances. Typical radiological findings are osteosclerosis, coarsened trabeculae, compact bone thickening and ossification of tendon and ligament attachment points. However, radiological changes range from diffuse osteosclerosis to diffuse osteopenia including features of secondary hyperparathyroidism, rickets, and osteomalacia.[3,9,15-17] Thus, Lian and Wu[18] reported that osteopenia of the long bones, such as the distal part of the radius, appeared before any other changes in the development of endemic skeletal fluorosis.

Krishnamachari reported severe osteoporosis of the distal part of the femur, the proximal part of the tibia, and the proximal part of the fibula along with rarefaction of the metacarpal bones as some of the most striking radiologic features of fluorosis.[6] Mithal *et al*[3] also studied the spectrum of radiological findings in endemic fluorosis.[3] They demonstrated a wide variety of radiological patterns such as coarse trabecular pattern, axial osteosclerosis with distal osteopenia, and diffuse osteopenia. In 30% of the patients no radiological anomalies were detected. Furthermore, in their series, three children also had some features

resembling rickets in the form of fraying of metaphyses and apparent widening of the GP cartilage. We also observed some features resembling rickets such as the relative widening and late partial fusion of GP in rats that had been exposed to high levels of fluoride.

Several factors influence the type of bone changes. These include the dose and duration of fluoride exposure, hormonal status, age and dietary habits. The different presentations of endemic fluorosis are probably related with different combinations of these factors.[3] It is well known that fluoride stimulates alkaline phosphatase activity.[13,19] Roholm[20] suggested that small doses of fluoride stimulate bone growth and calcification and large doses cause bone atrophy. Mithal *et al*.[3] found that excessive fluoride with reasonable calcium intake leads to osteosclerosis, while a calcium deficient diet together with fluoride excess can produce osteopenia.[3] In some studies,[21] excess fluoride was observed to cause secondary hyperparathyroidism. Parathyroid hormone causes growth plate to widen,[4] and the wider growth plates detected on radiographic examination of high fluoride treated rats can therefore be seen as a consequence of fluoride-induced hyperparathyroidism.

The effects of fluoride on bone have been studied extensively by both radiological and histopathological methods. However, knowledge of how fluoride affects GP is still incomplete. In our experimental study using histopathological and radiological methods, we observed that excessive fluoride intake caused a delay in the fusion of the GP. However, further studies are required to explain the mechanism of the effects of fluoride exposure during growth.

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