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EFFECT OF ENDEMIC FLUOROSIS ON THE MORPHOLOGY AND X RAY DIFFRACTION PATTERN OF GOAT BONE APATITE

(With One Table and 19 Figures)

By

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تأثير التلوث البيئى بالفلورين على طبيعة العظام وتشتت الأشعة السينية للبلورات العظمية في الماعز

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تعد إصابات العظام ببعض الملوثات الصناعية الكيميائية من أخطر الآثار الناجمة عن التعرض المستمر لهذه الملوثات · ويعد التسم المزمن بالفلورين واحد من أهم هذه الملوثات الصناعية · وقد هدفت هذه الدراسة إلى توضيح العلاقة بين الفلورين بمستويات المختلفة في عظام الماعز المتعرض للتلوث البيئى بالفلورين والتغيرات التكوينية الدقيقة للنسيج العظمى في مراحل العمر المختلفة (أجنة ـ الرضيعة ـ البالغة ـ المصابة بآفات عظمية) بالمقارنة بعظام حيوانات بعيدة عن مناطق التلوث كضابط للتجربة · وقد تم إجراء هذه الدراسة على الماعز البلدى المتواجد بمنطقة عزبة الأكراد الواقعة جنوب مصنع السوبر فوسفات نحو ١/٢ كيلو متر · وقد إشتملت هذه الدراسة على عشرة حيوانات قسمت إلى ثلاث مجموعات على حسب العمر وظهور الآفات التشريحية للعظام بالإضافة إلى خمسة أجنة تم أخذها في شهرها الرابع · ومقارنتها · جميعاً بمجموعة من أربعة حيوانات كضابط للتجربة · تم فحص هذه العظام بالمجهر ذو الضوء المستقطب كما تم تعيين تركيز الفلورين بها مع قياس حجم البللورات العظمية عن طريق قياس تشتت أشعة أكس ، وقد أظهرت نتائج هذا البحث عن وجود علاقة طردية بين معدل تركيز الفلورين وحجم البللورات العظمية ، كما قد تم تسجيل التغيرات في التركيب البللورى والغير بللورى لمادة العظام وتخلل المواد الغير بللورية في التركيب البللورى للعظام · ويتضح من هذه الدراسة مدى الخطورة الشديدة للتعرض المستمر للفلورين على العظام ·

SUMMARY

Examination of bone samples of goats from an endemic fluorosis locality revealed that crystallic apatite varies from a mixed particles, venous structures and compact zones extended allover the bone structure certainly in the inner layers of the cortical bone. The crystal index of examined bones (B values) were low in specific bone lesion, young and adults compared with foetal and control groups. The largest crystal size was detected in bone lesions and gradually decreased in adults, youngs, foeti and control bones respectively.

INTRODUCTION

Environmental pollution is one of the most hazardous agents to animal and human beings health status. Industrial pollutants added more hazzrds to environment. Fertliser manufacture, aluminium, brick, ceramics, steel and compustion of coal industries release fluoride to environment in the form of gasseous hydrogen fluoride, silicon tetrafluoride and fluoride particulates. A cattle disease resembling "Osteomalacia" near by an Italian superphosphate factory was investigated by BARTOLUCCI (1912). Spontaneous fracture in long bones and ribs had been recorded by GRÜNDER (1972) around aluminium factory in West Germany in cattle and SEDDEK (1988) around superphosphate producing factory in Egypt in goats. The changes were observed experimentally by MILHAUD et al. (1984) in suckling lambs. Non inflammatory swollen extrimities with spontaneous fractures in cattle was manifested in Brazil (CORREA et al., 1986). ERGUN et al. (1987) detected fluorosis in sheep and human in Turkey.

The apatite crystals in skeletal tissues are extremely small and plate like which become progressively sharper and better resolved as the percent of fluoride increased. X-ray diffraction investigations of series of adult human bones which varied in fluoride content (0.03-0.8%) were the first studies to provide an evidence of changes in crystal morphology with fluoride uptake (ZIPKIN et al., 1962 and POSNER et al., 1963). Small-angle X-ray diffraction analysis indicated that the changes in crystallinity with flucride are primarily owing to changes in crystal size (ENES et al., 1965).

The morphological changes of bone matrix and the crystal size of each case of different ages of goats in relation to their fluoride contents determined.

MATERIAL and METHODS

Ninteen middiaphysis metacarpal samples of bones were obtained from 14 goats and 5 foeti, from an endemic area of fluorosis (Ezbet El Akrad) which located 0.5 km away at South of the superphosphate plant of Manquabad, Assiut. Age and stages of endemic fluorosis were recorded (Table 1). El-Nokhaila village was chosen as control because it located 35 Kms to the south of the factory. The previous toxicological studies in this village, revealed no clinical signs, that could point to exposure to any hazardus substance (SEDDEK, 1988).

According to the method of FRY and TAVES (1970) bone samples were firstly examined physically and thin sections were inspected by means of polarised light microscope for morphologic changes in bone matrix. Bone samples were dried at 105°C overnight and then ground. Every sample of bone powder was devided into two portions. One was ashed at 600°C (6 hrs) for estimation of fluorides and ash %. The other portion was subjected to X-ray diffraction analysis. Fluorine was determined by means of fluoride, 94-09 attached to single junction references electrode model 90-01 fitted to expandable ion analyser EA 920, Orion research incorporated, Cambridge, U.S.A.

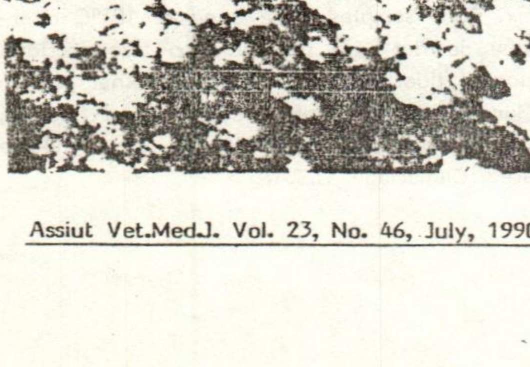
The powdered bone samples were subjected to Siemens D 501, (40 Kv/30 mA), Apparatus. X-ray diffraction patterns (Copper K & radiation) in order to determine the crystallographic changes. The degree of resolution of the principal X-ray reflections of bone apatite pattern was used as a "Crystallinity" of each sample. In this sens "Crystallinity" includes the effect of crystal size and crystal imperfection since both contribute to the broading of X-ray diffraction maxima. The X-ray pattern was assigned "Crystallinity values, "B". As the values of "B" decrease, the crystal size increases. The applied method was described by POSNER et al. (1963).

RESULTS

The examination of bones by the polarised light revealed various gross changes according to the status of fluorine content and age. The crystalic apatite varies from a mixed particles, venous structures and compact zones extent allover the bony structures (Figs. 1, 2, 3). The amorphus substances differ in quantity and conversily related to the well developed apatite crystals. The great difference was present in the inner layer of the cortical bone (Fig. 4). It was found that the most outer layers had no changes where a well developed crystallic appatite was present except in cases of bone lesion surfaces which revealed no covering layer of a well formed apatite.

The obtained crystal index was recorded in table (1) and designed in Fig. (5). It is apparently that the means of "B" values are low in specific bone lesions (1.19 °2Ø), youngs (1.25 °2Ø) and adults (1.24 °2Ø) where foetal and control bone samples had a higher crystallinity index of 1.48 and 1.36 °2Ø respectively (Figs. 6,7,8,9,10). The crystal volume was correlated conversly to "B" where the largest crystal volume was detected in bone lesions and declined in vlaues in adults, youngs, foeti and control bones. the crystal volume was recorded as 0.622, 0.740, 0.741, 0.778 and 0.678 X 10^{-6} cm and fluorine content as 1042, 3341, 8426, 13430 and 813 ppm for foeti, young, old, lesion and control bones respectively (Table 1).

Statistical analytical studies was calculated according to KALTON (1967).



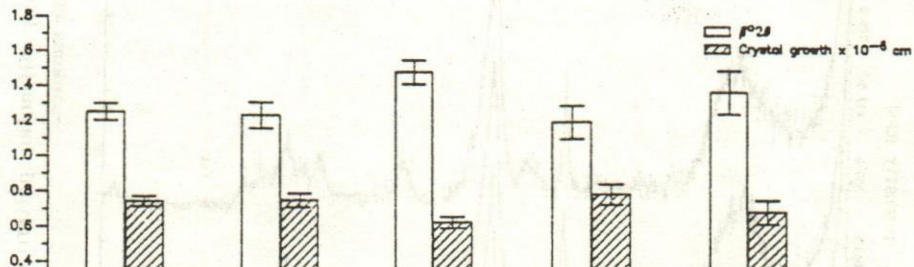


Fig (5): Crystal index and growth of the investigated bones.

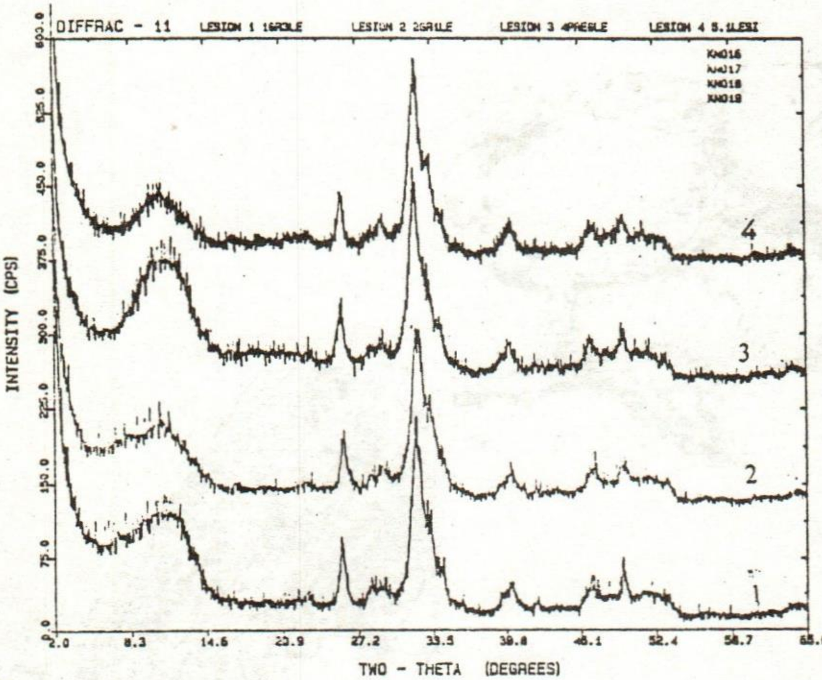


Fig.(6): X-ray diffraction pattern of four goats bone lesions.

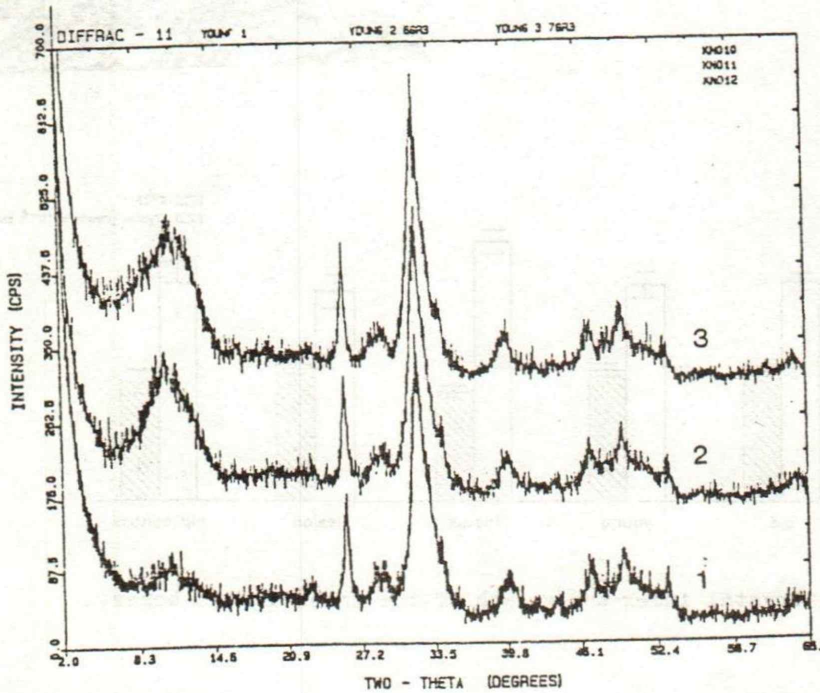


Fig. (7): X-ray diffraction pattern of three young goats bone samples.

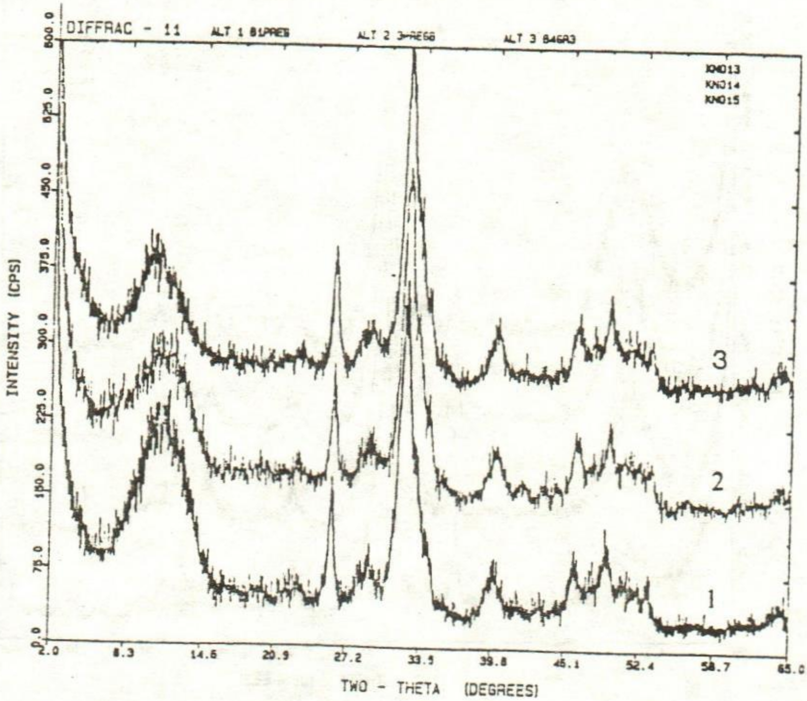


Fig. (8): X-ray diffraction pattern of three old goats bone samples.

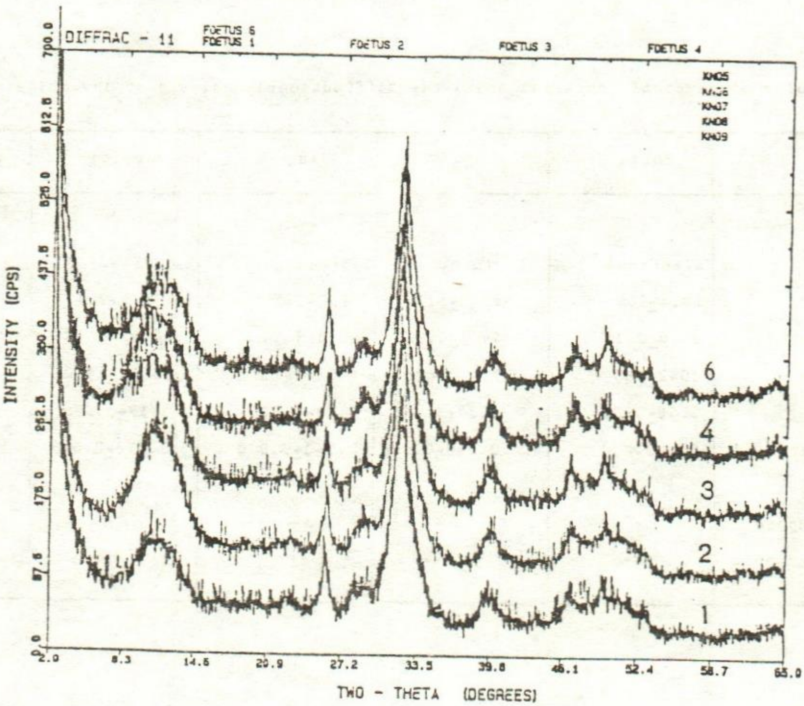


Fig. (9): X-ray diffraction pattern of five foetal bone samples.

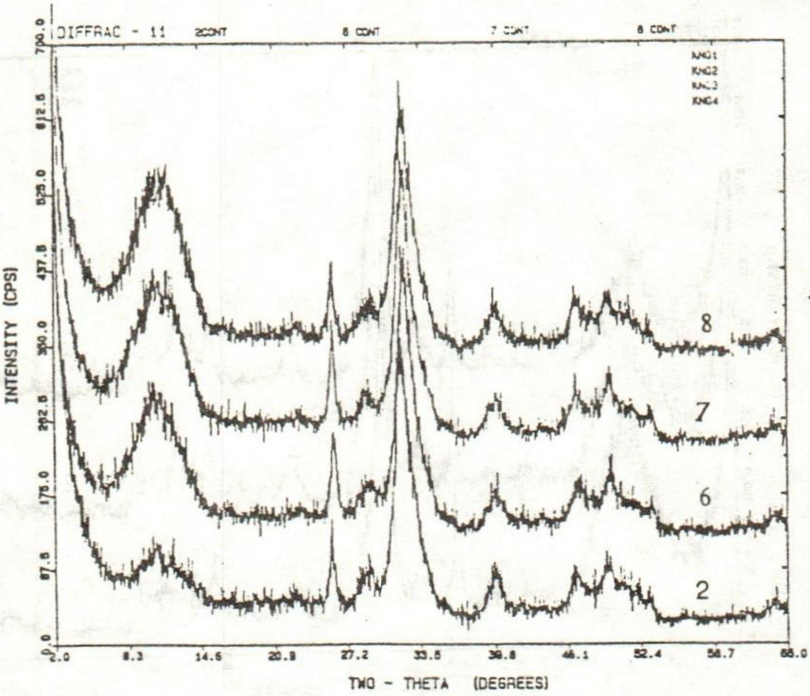


Fig. (10): X-ray diffraction pattern of four old control samples.

Table(1): Results of physical, chemical and x-ray diffractional analysis of investigated bones.

	Foeti	Young	Old	Lesion	Control
No.of cases	5	3	3	4	4
Age	4(months)	2-3(months)	2-3(years)	2-3(years)	2-3(years)
Dry matter %	59.4±1.4	65.0±1.3	87.0±2.1	76.9±2.3	91.86±1.5
Ash %	60.9±0.15	59.2±0.26	66.4±0.24	60.92±0.38	65.7±0.07
Fluorine ppm.	1042±380	3341±460	8426±1980	13430±1630	813±139
B°2 θ	1.48±0.069	1.25±0.076	1.24±0.050	1.19±0.096	1.36±0.025
Crystal growth $\times 10^{-6}$ cm.	0.622±0.033	0.740±0.040	0.741±0.030	0.778±0.059	0.678±0.068
Apatite incidence	+	+	++	+	+++
Amorphous incidence	+++	+++	++	+++	+

DISCUSSION

Bone examination is considered one of the most important aids in diagnosing cases of endemic fluorosis. Recent reviews on the crystal habit of bone apatite provide little informations on the effect of fluoride on the crystallinity of animal bone.

The findings in the present study revealed that the increase in amorphus particles related linearly with the increase in fluorine content of bones. The amorphus substances differ in quantity and conversely related to the well developed apatite crystals. This finding could be explained through the chemical analytical records of ZIPKIN et al. (1960) who found an increase in Magnesium levels of fluorotic bones. and SEDDEK (1988) who found too an increase in Mg, Zn, Cd, pb, P in the examined fluorosed goats. The formation of more amorphous in relation to the crystalic apatites could be attributed to the forementioned elemental increase. Therefore, attention is best attracted to possible substitutions and defeciencies within bone matrix and the apatite crystal structure where substitution of strontium, stannous, Molybdenum, cadmium, lead, rare earths, sodium, and Magnesium for calcium substitution and of vanedium, Arsinic and sulphur for phosphorus was postulated by BRUDEVOLD and SOREMARK (1967).

The crystal index obtained in our study was low in specific bone lesions (1.19) while its parameter elevated for youngs (1.25), adults (1.24), control (1.36) and foeti (1.48) 2°Ø respectively. The fluorine analytical studies of examined bone samples revealed high levels for both young, old and bone lesions. Foeti and control bones showed its low levels. Our results indicated a change in both bone matrix amorphous contents and the crystalic size index "B" at levels of fluoride of 3300 ppm of youngs. The record of SHUPE (1960) indicated that the definite microscopical abnormalities observed in those bones containing 4000 ppm or more fluorine could be partially attributed to the change in the chemical structure of bone matrix.

The results of crystal index "B" obtained in our study were supported by ZIPKIN et al. (1962) who found an increased resolution of X-ray diffractional powder pattern of a sample of iliac crest containing 0.873% fluoride (Ash bases) compared with a similar sample containing 0.224% fluoride. The "B" value were 0.5, 0.8 °2Ø respectively.

The crystallinity of bone apatite increased (i.e.B values decreased) as the fluoride contents of the bones increased. The author recommended that fluroide may increase the size and/or reduce the strain within the bone crystalities producing a decrease of the effective surface area per unit mass of bone and therefore reducing the reactivity of bone apatite. The reason for this highly and isotropic response to fluoride is not clearly understood. It is possible that fluoride has an effect on crystal growth kinetics (POSNER, 1963). Such a direct action, however, is not supported by EANS and MEYER (1978). It is also possible that the observed changes with Fluorides may reflect the importance of the organic matrix in controlling crystal growth. It has been proved from our results that not only organic matrix but also the mineral status of matrix may play a part in the response of crystallinity (Size and perfection) to fluorides.

The results of these study clarified the correlation between bone lesions, morphological changes of bone matrix, crystal size and fluoride content in different ages of goats in case of endemic fluorosis.

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