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Endemic fluorosis — a model for studies examining the effect of fluoride on bone

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The use of fluoride in the treatment of osteoporosis is complicated by concerns about the integrity of the newly formed bone, as well as the fact that not all patients respond to the drug. In an attempt to broaden insight into the varied skeletal response to fluoride exposure, we studied (clinical, dietary analysis, radiology, biochemistry and genetic markers) all 114 permanent inhabitants of an endemic fluorosis area. Unequivocal radiological evidence of osteofluorosis was present in 26% of subjects, while 48% had normal skeletal radiological results and 4% were found to be osteopenic. Twenty-two per cent were unclassifiable. Individuals with fluorosis were older and predominantly male. Although musculoskeletal symptoms occurred more frequently in patients with osteofluorosis, these subjects did not have an increased fracture prevalence and were, in fact, found to have a higher metacarpal as well as femoral cortical bone mass. In subjects with osteofluorosis, the average fluoride intake and residency in the area were similar to those of unaffected Kenhardt individuals, implying that there are causative factors other than fluoride exposure. Moreover, renal excretion of fluoride was comparable, as was the average energy, protein, mineral and alcohol intake. Mean serum calcium, phosphate, alkaline phosphatase, parathyroid hormone, calcitonin and 25-hydroxyvitamin D levels were unremarkable and similar in subjects with and without osteofluorosis. However, a family tree analysis of the population revealed that 4 very closely related subjects had osteopenia, which suggests that a genetic predisposition may at least partially explain why the skeletal response to fluoride is not determined by fluoride exposure alone.

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The use of fluoride in the management of osteoporosis has been a source of controversy since it was first suggested by Rich and Ensinck[1] more than 3 decades ago. Clinical studies have produced extensive evidence to support the contention that fluoride is one of the most effective agents available to increase spinal bone density over a sustained period.[2] Treatment is, however, not without pitfalls and a definite proportion of patients (15 - 30%) do not respond to the drug.[3] It has been suggested that failure to respond may be due to intestinal malabsorption and/or impaired skeletal uptake of fluoride.[4] This does not, however, explain the phenomenon, since responders and non-responders have been shown to have comparable blood fluoride levels during treatment. Apart from a recent report documenting higher urinary fluoride levels in responders,[5] no clear reason for this apparent individual sensitivity has yet been identified.

In osteoporotic patients responding to fluoride therapy, the beneficial mitogenic effect is dose-dependent and linear with time during continuous administration.[6] Fluoride has been shown to increase the birth rate and number of osteoblasts, the rate of bone formation[7] and the synthesis of alkaline phosphatase and osteocalcin.[7,8] However, a 'toxic' effect at the individual osteoblastic cell level, as evidenced by a reduction in osteoblastic activity and a delay in mineralisation, has been well established.[9] Moreover, sodium fluoride has been associated with a painful lower-extremity syndrome believed to represent an imbalance between matrix production and mineralisation, leading to micro-fractures.[10] Some studies have suggested that fluoride increases trabecular connectivity and strength,[11] whereas others reported the failure to induce *de novo* generation of trabeculae or the restoration of connectivity.[12] Effects on cortical bone are less marked, and it has been suggested that fluoride-induced remodelling could increase intracortical porosity and may enhance the risk of future fracture.[13]

Recently, Riggs and co-workers[14] reported a 30% increase in spinal bone mass, but a significantly increased rate of non-vertebral (including hip) fractures after fluoride therapy. Although these findings have since been refuted by several studies and generally ascribed to differences in fluoride bio-availability and/or the dose employed,[15] some concern about fracture prevalence in fluoride-treated patients still remains.

In an attempt to gain a better understanding of the underlying reasons for the varied skeletal response to fluoride exposure, we studied an area of endemic fluorosis which is situated in the Kenhardt district of the Northern Cape Province.

Patients and methods

All procedures for this study were approved by the Institutional Review Board and Ethics Committee of the Stellenbosch University Medical School and informed consent was obtained from each subject. All the permanent inhabitants (50 males and 64 females, aged 6 - 70 years) of an isolated community resident in the endemic fluorosis district of Kenhardt, situated some 700 km north-west of Cape Town, were studied. This population is of low socio-economic and educational background. The fluoride content of the only drinking water supplying this village (bore-holes) was shown to be 6.2 mg/l, which greatly exceeded the accepted safe level of 1 mg/l. Individuals were subjected to a detailed history and full clinical examination, which took due cognisance of previously reported manifestations associated with excessive fluoride ingestion, e.g. bone or joint pain and tenderness, spine or long-bone deformities, arthritis, calcifications.[16] None of the subjects had previously received any treatment for bone diseases. A dietary analysis of all subjects, performed by a qualified dietician, included a quantified food frequency questionnaire on habitual food intake during the month preceding the study. Portion sizes were standardised by the local Research Institute for Nutritional Diseases and the reproducibility of data obtained from the questionnaire was verified before use in this study. Current as well as previous alcohol and nicotine consumption were also documented.

The radiological evaluation of each patient included a postero-anterior and lateral view of the chest, thoracic and lumbar spine, the pelvis (including the proximal third of each femur) and both hands. Appendicular bone mass was quantified by radiogrammetry of the second metacarpal, the Singh index and measurements of the calcar femorale.[17] Previously reported radiological manifestations of osteofluorosis,[16] such as increased bone density, coarsened trabeculations, hypertrophic changes, ligamentous calcifications and signs of secondary hyperparathyroidism or osteomalacia, were also documented. Subjects with osteofluorosis were further divided into those with moderate and those with severe disease, based on the degree of bony sclerosis. All radiographs were evaluated by two independent observers and skeletal radiology formed the basis of this comparative study between patients with and without fluorosis.

Fasting serum calcium (total), phosphate, alkaline phosphatase (ALP), creatinine, electrolytes and liver function profiles were determined by SMAC (Technicon Autoanalyzer, Basingstoke, UK). The serum calcium concentration was corrected to a plasma albumin level of 40 g/l (0.02 mmol/l calcium per g/l albumin). The serum magnesium level was measured calorimetrically (Bio Merieux, England). A double antibody sequential competitive radio immunoassay kit was used to measure circulating calcitonin (Diagnostic Products Corporation, USA), and for immunoreactive parathyroid hormone a C-terminal assay with the antibody directed at the 65 - 84 sequence of human iPTH was used (Immuno Nuclear Corporation, USA). Serum 25-hydroxyvitamin D was determined by a competitive protein-binding assay, employing a rat kidney receptor, after rapid lipid extraction and preliminary purification of serum on a C18 Sep-Pak cartridge, followed by purification on a silica Sep-Pak with hexane isopropanol.[18]

Urinary calcium and creatinine were measured with an Astra system (Beckman, USA). An ion-selective electrode (Orion Model 94-09; Orion Research Inc., Cambridge, USA) was used to measure fluoride in the drinking water and urine.

A family tree analysis of the entire population was performed and a limited number of specific genetic markers were determined. HLA typing was performed by means of a standard microlymphotoxicity technique. The ABO, MNSs, Rh, and FY blood groups were tested using standard serological methods.

Statistical analysis

Results were analysed by means of a two-sample *t*-test or analysis of variance when more than two groups were compared. Lod scores were estimated with the LIPED computer programme.

Results

The fluoride content of the drinking water was found to be 6.2 mg/l, which is six times higher than the accepted safe level of 1 mg/l. These high fluoride levels did not, however, uniformly produce radiological bone changes in the population.

Radiology

Skeletal radiology, assessed by two independent observers without prior knowledge of the subject matter, formed the basis for further comparison between normal, fluorotic and osteopenic subjects. Clear radiological evidence of osteosclerosis (causes other than fluoride excess excluded, e.g. Paget's disease of bone, skeletal dysplasias) was present in 26% of subjects, while 48% had normal skeletal radiology. Four per cent of the population was osteopenic with evidence of vertebral translucency and deformities (biconcavity, wedging or compression of more than 20%),[19] while the remaining 22% could not be classified with certainty as either normal or affected; these probably largely represented subjects with early fluorosis, but were not included for further comparison. The 5 osteopenic subjects were all female, and 4 were closely related, viz. a mother aged 65 years, two daughters (44 and 46 years) and a granddaughter (24 years). With the exception of rib fractures in 2 osteofluorotic patients and vertebral fractures in osteopenic individuals, no signs of skeletal fractures or osteomalacia were detected in any subject. Osteosclerotic individuals were, furthermore, classified as having either moderate or severe disease. Ligamentous calcification, hypertrophic changes and bony overgrowth were almost entirely confined to those with severe osteosclerosis.

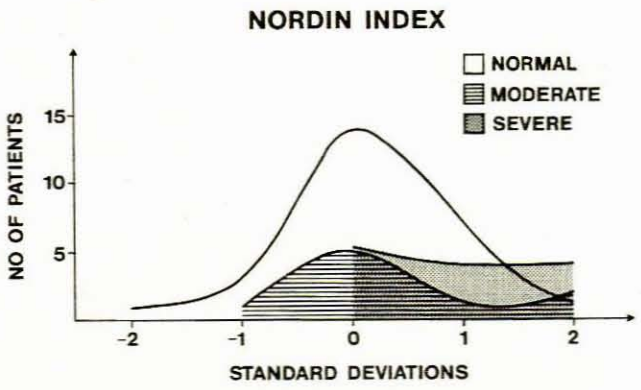

Fig. 1. Metacarpal cortical thickness (MCT) or Nordin index of patients with normal radiographs, moderate and severe osteofluorosis. The MCT of subjects with severe osteofluorosis differed significantly ($P < 0.01$) from that of individuals with normal radiographs.

We have previously established normative radiogrammetric data for metacarpal cortical measurements in our local populations.[20] In the present study individuals with moderate and severe osteofluorosis were found to have a higher cortical bone mass than their unaffected counterparts who displayed a regular distribution of metacarpal cortical thickness (Fig. 1). Normal calcar femorale measurement values for children are not available and evaluation of this parameter and the Singh index were therefore limited to the adult study population. Assessment of the Singh index and thickness of the calcar femorale did not differ markedly between subjects with normal and moderately sclerotic radiographs but both were significantly higher in patients with severe osteofluorosis (Table I).

Table I. Singh index and thickness of the calcar femorale in adults with normal, fluorotic or osteopenic skeletal radiology

	Normal radiographs	Moderate osteofluorosis	Severe osteofluorosis	Osteopenia
Calcar femorale (mm)				
Males	6.8 ± 0.5	7.0 ± 0.5	8.8 ± 0.2	
Females	6.1 ± 0.3	5.9 ± 0.5	8.4 ± 0.9	
Total	6.2 ± 0.3	6.4 ± 0.3	8.5 ± 0.3*	4.9 ± 0.4†
Singh index (scored from 1 to 6)				
Males	5.3 ± 0.3	5.6 ± 0.2	6.0 ± 0	
Females	5.2 ± 0.2	5.6 ± 0.2	6.0 ± 0	
Total	5.2 ± 0.1	5.6 ± 0.1‡	6.0 ± 0¶	4.4 ± 0.3§

Data are presented as the mean ± SEM.
* Normal v. severe osteofluorosis ($P < 0.0001$).
† Normal v. osteopenia ($P < 0.05$).
‡ Normal v. moderate osteofluorosis ($P = 0.0184$).
¶ Normal v. severe osteofluorosis ($P < 0.0001$).
§ Normal v. osteopenia ($P = 0.0054$).

Clinical data

Clinical and biochemical data on all 29 individuals assessed with radiological osteofluorosis were compared to those of the 55 subjects with normal skeletal radiology.

Affected individuals were older and predominantly male (Table II), although children as young as 6 - 8 years were found to be severely osteosclerotic. In subjects with fluorosis the average fluoride intake, residency in the area and years of exposure were similar to those of unaffected controls, implying that causative factors other than fluoride exposure alone exist (Table II). Musculoskeletal symptoms (e.g. bone pain, arthralgia, joint pain) and clinical signs (bone tenderness, arthritis, synovitis, kyphoscoliosis, genu varum, genu valgum, tibial bowing) were more prevalent in patients with osteofluorosis, especially the older subjects with severe disease, although a history of skeletal fracture or the presence of dental fluorosis occurred as commonly in subjects with normal skeletal radiology.

There were no significant differences in the daily intake of nutrients (expressed as a percentage of recommended daily allowance) between normal and fluorotic subjects. The calcium intake was low, but comparable between groups, while all other nutrients were consumed in sufficient quantities (Table III).

Table II. Clinical data

	Normal radiographs (N = 55)	Osteofluorosis (N = 29)
Age (yrs)	31 ± 4	40 ± 6
Gender (male/female)	1:2	7:3
Fluoride exposure		
Fluoride intake (mg/day)	9.1 ± 0.7	9.9 ± 0.8
Residency in area (% of lifetime)	69 ± 5	71 ± 5
Total years of exposure (yrs)	22 ± 3	28 ± 4
Clinical signs and symptoms (% of subjects affected)		
Dental fluorosis	100	100
Musculoskeletal symptoms	23	100
Clinical skeletal abnormalities	17	77

Data are presented as the mean ± SEM.

Table III. Dietary history

	Dietary intake (% of RDA)	
	Normal radiographs	Osteofluorosis
Calcium	49 ± 4	55 ± 6
Phosphorus	98 ± 8	129 ± 17
Protein	106 ± 8	105 ± 10
Energy	157 ± 11	160 ± 12

Data are presented as the mean ± SEM.

Biochemistry

The serum levels of corrected calcium, phosphate and magnesium were normal and comparable between groups, while the mean total serum ALP level was modestly raised in all subjects, more pronounced in those with severe osteofluorosis, although this did not reach statistical significance (Table IV). Likewise, the urinary excretion of calcium and fluoride were similar in subjects with and without osteofluorosis.

Calciotropic hormones

The concentrations of the calciotropic hormones, parathyroid hormone (PTH) and calcitonin, were similar, but 25-hydroxyvitamin D levels were significantly, albeit modestly, lower in subjects with severe osteofluorosis (Table IV).

Genetic markers

A family tree analysis of the entire population revealed that individuals belonged to one of five large families. Osteofluorotic individuals were more prevalent in some families and the genetic model proposed was one of dominant inheritance with incomplete penetrance. Furthermore, of the 5 individuals who were radiologically osteopenic, 4 were closely related: a mother, 2 daughters and a granddaughter (Fig. 2). Linkage of a putative locus for susceptibility or resistance to the development of fluorosis could be excluded at the HLA locus and was indeterminate at the other marker loci examined.

Table IV. Biochemical data

	Normal radiographs (N = 55)	Osteosclerosis	
		Moderate (N = 18)	Severe (N = 11)
Serum			
Corrected calcium (mmol/l)	2.30 ± 0.01	2.36 ± 0.01	2.34 ± 0.01
Phosphate (mmol/l)	1.16 ± 0.07	1.14 ± 0.14	1.18 ± 0.12
Magnesium (mmol/l)	0.83 ± 0.07	0.82 ± 0.06	0.84 ± 0.07
Alkaline phosphatase (U/L)	104 ± 5	104 ± 6	133 ± 16
Creatinine (µmol/l)	85 ± 2	77 ± 5	82 ± 4
Parathyroid hormone (pmol/l)	49.5 ± 3.7	45.9 ± 5.1	46.0 ± 2.9
Calcitonin (pmol/l)	7.0 ± 0.6	6.5 ± 0.3	6.5 ± 0.3
25-hydroxyvitamin D (ng/ml)	22.4 ± 0.5	21.9 ± 0.8	17.1 ± 1.7*
Urine			
Calcium/creatinine (mmol/mmol)	0.27 ± 0.01	0.29 ± 0.02	0.28 ± 0.02
Fluoride (µmol/l)	447 ± 38	451 ± 36	474 ± 61
Fluoride/creatinine (µmol/mmol)	52.7 ± 2.7	58.3 ± 2.8	57.5 ± 5.1

Data are presented as the mean ± SEM.
* Normal v. severe osteosclerosis, $P < 0.05$.

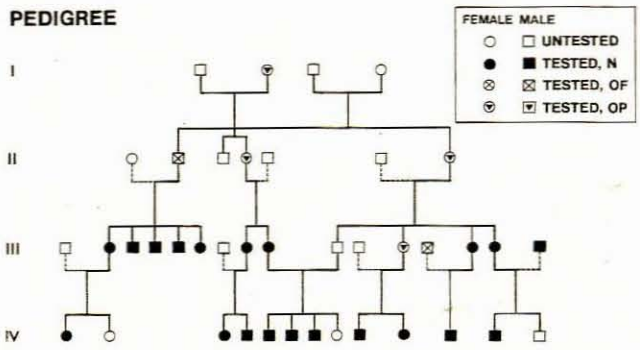

Fig. 2. Partial family tree which includes 4 of the 5 osteopenic subjects in the entire study population.

Discussion

We investigated the entire permanent population of an area of endemic fluorosis in order to highlight selected aspects of this condition and in an attempt to extrapolate conclusions to some of the clinical problems encountered in the treatment of osteoporotic patients with fluoride. Owing to the remote location of the area, osteodensitometric techniques to assess appendicular as well as axial bone mass could not be employed, and standard radiology formed the basis for comparison between normal, fluorotic and osteopenic subjects. The fluoride content of the drinking water was found to exceed greatly the accepted safe level of 1 mg/l, yet did not uniformly produce radiological bone changes. Despite the long-term excessive ingestion of fluoride in all the subjects, only 26% of the

population had clear radiologically detectable skeletal changes.

Clinical features were nonspecific, essentially confined to the musculoskeletal system, and not very helpful in predicting the presence of fluorosis in an individual subject. Dental mottling was present in all subjects, probably due to the fact that subjects were exposed to fluoride during the period of tooth formation. There was no association between the degree of dental and skeletal involvement. Clinical manifestations included bone pain, joint pain and swelling and bowing of the femora or tibiae. These findings were more prevalent in, although not exclusive to, subjects with severe fluorosis. Evidence of ligamentous and soft-tissue calcification, previously suggested to be common early features of osteofluorosis,[16,21] was only detected in cases of advanced disease in the present study. An increased radiological bone density was found to be the only typical early change.

Some of the more controversial aspects of fluoride therapy in osteoporosis concern the structural integrity of the newly formed bone — specifically the induction of a mineralisation defect — and the possibility that fluoride-induced remodelling could increase intracortical porosity with consequent cortical thinning and an increased fracture risk.[10-14] In this study, no radiological signs of osteomalacia were detected in any subject. Serum levels of calcium, phosphate, ALP and PTH were normal and similar in subjects with and without osteofluorosis. Moreover, fluorotic patients did not have an increased fracture prevalence and were, in fact, found to have an increased metacarpal as well as femoral cortical bone mass.

The varied skeletal response to fluoride exposure, previously documented in osteoporotic patients treated with this agent[5] and in endemic fluorosis populations,[21] was confirmed in our study. Conceivably, the underlying mechanism of this individual susceptibility could involve either differences in the pharmacobiology of fluoride and/or the skeletal response to the agent. It is known that the amount of bio-available ionic fluoride in the body depends on the quantity of ingested fluoride and its passive absorption from the gastro-intestinal tract. Fluoride follows only two post-absorptive pathways, viz. urinary excretion of the majority, and skeletal uptake of the remainder by incorporation into the hydroxy-apatite crystal lattice of bone. Calcified tissue is therefore virtually the only retention site for fluoride.[22] Although it has been suggested that failure to respond may be due to intestinal malabsorption of fluoride and/or its impaired skeletal uptake, little evidence exists to support this hypothesis.[23] It has been shown, in an area of endemic fluorosis, that the fluoride content of the drinking water correlated with the fluoride content of bone,[24] and Marcelli *et al*.[25] recently reported that the bone fluoride content was not significantly higher in responders than in non-responders after 2 years of fluoride therapy. In our study population the fluoride content of the drinking water was six times higher than the accepted safe level. In subjects with osteofluorosis, the average fluoride intake and duration of residence in the area were similar to those of unaffected controls, implying that causative factors other than fluoride exposure exist. Moreover, renal excretion of fluoride was similar in affected and normal individuals. The rapid uptake of fluorine-18 has been shown to occur in bone sites where calcium accretion is the most active.[26] Bone changes induced by fluoride may therefore depend not only on the dose received, but also on the level of bone turnover.[22] One could therefore assume that fluoride uptake may be higher in younger individuals with an active modelling (child) or remodelling (younger adult) skeleton. However, since fluoride can, in itself, stimulate bone formation, and thus calcium accretion, it is likely that it augments its own uptake.[27] In our study population, we found responders and non-responders throughout the entire age spectrum. In an endemic fluorosis area in Finland, males and females were affected equally,[24] while in a group of osteoporotic patients treated with sodium fluoride, males increased their bone mineral density significantly more than females (6.3% v. 3.8% per year) during the first 18 months of follow-up.[3] In the Kenhardt population men and women of all ages were affected, males having a higher prevalence of skeletal involvement.

A number of biochemical changes have been noted during treatment with fluoride. Serum ALP and osteocalcin levels tend to increase and although intestinal calcium absorption remains unaltered, a decline in its urinary excretion has been demonstrated, a finding attributed to the skeletal retention of calcium induced by fluoride.[28] Recently Stamp *et al*. reported that non-responders to fluoride therapy were characterised by low serum ALP and phosphate, and increased serum PTH and urinary calcium levels.[29] In the Kenhardt population mean serum ALP was higher in patients with severe fluorosis, but serum calcium, phosphate, PTH and urinary calcium excretion were comparable.

Previous reports in patients,[30] as well as experimental animal models,[31] have suggested that a low dietary intake of calcium may predispose to the development of fluorosis. These findings could not be confirmed in our study, where the calcium intake was low in the entire population, responders and non-responders alike. Serum calcium and urinary calcium excretion were also similar in all groups. Serum 25-hydroxyvitamin D levels were modestly, albeit significantly, lower in subjects with severe osteofluorosis but identical in those with moderately osteosclerotic and normal skeletal radiographs. Mean 25-hydroxyvitamin D levels in all groups were however well within the normal range. Although we cannot rule out abnormalities of calcium homeostasis as a concomitant causative factor, this possibility would seem unlikely.

Perhaps the most interesting finding of this study was the observation that the individual susceptibility of skeletal tissue to fluoride may have a genetic basis. Despite exposure to excessive amounts of fluoride, 5 individuals were found to be osteopenic, 4 of whom were very closely related. This observation suggested that the skeletal response to fluoride may be partly genetically determined. The possibility that the osteopenia could have been determined by a separate gene (e.g. for osteogenesis imperfecta or the vitamin D receptor) can, however, not be excluded. Linkage of a putative locus for susceptibility or resistance of the skeleton to fluoride could not be established by a limited number of genetic markers employed in this study. We conclude that a genetic basis for the varied osteoblastic response to fluoride exposure may exist and deserves further study.

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