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MINERAL CONTENT OF NONFLUOROSED AND FLUOROSED BONE - AN IN VITRO STUDY

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ABSTRACT

Aim: The literature on effect of fluoride on dental caries is well discussed in contrast to periodontal tissues. However a recent review has explored an epidemiological association between fluorosis and periodontal disease, but also the influence of fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non fluorosed teeth. There is a scarcity in literature dealing with effect of fluorosis on biological tissues like bone. Alveolar bone which is an integral part of periodontium similar to extremities has not been studied for mechanical, histologic and mineral aspects of fluorosed bone. Hence the aim was to study the histology of fluorosed and nonfluorosed femoral bone. **Material and methods:** A total of 24 healthy nonfluorosed and fluorosed bone (femur) specimens were collected to assess and compare the histology of fluorosed versus non fluorosed bone using light microscope. **Results:** Cellularity of cortical and cancellous bone was found to be statistically significant in nonfluorosed group (10.72±4.10, 8.74±2.34) when compared to fluorosed group (6.61 ±3.31, 5.69±1.31) respectively. Trabecular density of bone: Trabecular density was same in both nonfluorosed and fluorosed bone [statistically non significant, p= 0.615]. However trabeculae were thick in nonfluorosed bone and short and thin in fluorosed bone.

KEYWORDS: Dental fluorosis, periodontitis, mineral content, femoral bone.

INTRODUCTION

Fluorine is a common element in the earth's crust and is an essential element for the calcification of bones and teeth. Fluoride ion has played a major role in dramatically reducing dental caries over past 40 years. Excessive systemic exposure to fluoride can lead to disturbances of bone homeostasis, enamel development [dental/ enamel fluorosis] and mineralization. The severity of fluorosis on periodontal hard and soft tissues is dose dependent and also depends on timing and duration of fluoride exposure during development. (O. Fejerskov et al 1994).

Although fluorosis is a global and national problem, there are minimal researchers who are investigating the role of fluoride on periodontal tissues which increases the susceptibility to periodontal disease because of the alteration produced in both hard and soft tissues of periodontium. (K L Vandana 2014).

The literature on effect of fluoride on dental caries is well discussed in contrast to periodontal tissues. However, fifteen years of research and a recent review by Vandana K L has presented an epidemiological association between fluorosis and periodontal disease and also the influence of fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non fluorosed teeth. There is a scarcity of literature dealing with fluorosis effect on biological tissues like bone and cementum.(K L Vandana 2014).

Mineral aspects of fluorosed bone have been studied scantily. The mineral content and histologic aspects of fluorosed bone was studied by AK Susheela and Mohan Jha in an experimental rabbit study by giving high doses of sodium fluoride to assess the organic and inorganic constituents of bone and it was found that there was increased zinc, calcium, magnesium concentration and reduced collagen biosynthesis.(A.K Susheela and M Jha 1981) However, human bone from endemically fluorosed area have not been assessed so far.

The mineral content which may be different in fluorosed bone would influence the pathogenesis of periodontal disease and /or outcome of periodontal treatment. Even the orthodontic treatment which depends on bone quality would be affected fluoride induced changes in alveolar bone. The fracture reduction treatment and different bone pathologies may be influenced by fluoride induced changes.

Hence, the comparison of fluorosed versus nonfluorosed bone is a new area of interest in fluorosis research. Medline search using keywords fluorosed and nonfluorosed bone does not reveal much data. So present study aims to find out changes in mineral content of fluorosed versus nonfluorosed bone.

MATERIALS AND METHODS

A total of 24 healthy nonfluorosed and fluorosed bone (femur) samples were collected from orthopaedic section of S. S. Institute of medical sciences, Davangere. Subjects with age group of 35 to 55 (for bone) years of both the sexes were included respectively. Written consent was taken from all subjects and ethical clearance was obtained from the Institutional review Board (IRB; Ref No.CODS/ 2184) of College of Dental Sciences, Davangere, Karnataka according to Rajiv Gandhi University of Health Sciences, Karnataka protocols.

The bone samples were required to meet the following inclusion criteria: Bone specimens from systemically healthy patients; Fluorosed subject selection was based on following criteria – subjects who lived in the endemic water fluoride area for 5 to 10 years consuming water with fluoride levels above 1.2 to 3 ppm (Davangere water fluoride levels 0.2 mg/l to 2.41 mg/l), Subjects with mottled tooth enamel i.e, dental fluorosed stains assessed with the scores C, D E, F of Jacksons simplified fluorosis index (1974); Bone specimens were obtained from subjects who underwent surgical intervention following fracture due to trauma (accident) where in part of the bone (femur) was to be removed. The above criteria used for assessment of fluorosed teeth was considered to select the bone specimens. The exclusion criteria was Subjects with any metabolic bone disorders (hyperparathyroidism, pagets disease, hypophosphotasia) and infectious diseases. Sample size was 11.72 using $n= z^2 \sigma / (x_1 - x_2)^2$.

Procedural steps

Collection of bone specimens

Healthy nonfluorosed and fluorosed bone were collected and stored in bottles containing 10 % neutral buffered formalin. (Fonseca AA et al 2008).

Assessment of mineral content of bone

Each bone specimens were sectioned to 5mm x 5mm thickness. Energy dispersive spectroscopy (EDS - Genesis, FEI Quanta 200 high resolution scanning electron microscope) was used to identify the mineral content of bone. Interaction of an electron beam with a sample target produces a variety of emissions, including x-rays. An EDS detector was used to separate the characteristic x-rays of different elements into an energy spectrum, and EDS system software was used to analyze the energy spectrum in order to determine the abundance of specific elements. EDS was used to find the chemical composition of materials down to a spot size of a few microns, and to create element composition maps over a much broader area. Together, these capabilities provide fundamental compositional information for a wide variety of materials.

The minerals assessed were fluoride, calcium, phosphorous, magnesium, zinc, potassium, carbonate.

Statistical analysis

The data obtained from mineral content assessment was subjected to statistical analysis using SPSS 17.0. Comparision between groups was done using Unpaired t test. P value < 0.05 was considered to be statically significant. NS (p>0.05) = not significant; HS(p<0.001) = Highly significant.

RESULTS

22 healthy nonfluorosed and fluorosedbone (femur) samples were collected to assess and compare mineral content (energy dispersive spectroscopy - EDS) of fluorosed versus non fluorosed bone.

The results of the study are interpreted in table 1&2.

Mineral content of cortical bone

In nonfluorosed cortical bone percentage of calcium, phosphorous was more (30.44±10.00, 10.43±4.48) as compared to calcium, phosphorous (22.56±6.88, 7.463±2.264 of nonfluorosed cortical bone respectively. In the fluorosed cortical bone the percentage of fluoride, sodium, magnesium, zinc, potassium, carbonate was (0.520±0.086, 0.104±0.134, 0.483±0.259, 2.905±0.731, 0.549±0.237, 53.62 ± 13.31) more than fluoride, sodium, magnesium, zinc, potassium, carbonate (0.006±0.018, 0.103 ±0.12, 0.35±0.15, 2.52±0.94, 0.54±0.21, 65.27±6.24) of nonfluorosed bone respectively. The percentage of calcium and carbonate (p = 0.05, 0.02) was significantly more in nonfluorosed cortical bone respectively

Mineral content of cancellous bone

The percentage of calcium, phosphorous, potassium, carbonate was (35.67±7.466, 12.86±4.10, 0.646±0.266, 28.98± 8.00) more in nonfluorosed group than calcium, phosphorous, potassium, carbonate (27.29±8.00, 9.55±2.666, 0.608±0.426, 47.28+ 10.52) of fluorosed group. The percentage of sodium, magnesium, zinc, fluoride was (0.106±0.122, 0.263±0.195, 2.32 ± 0.71, 0.084±0.089) more in fluorosed group than sodium, magnesium, zinc, fluoride (0.105±0.110, 0.238±0.146, 2.288±0.945, 0.083±0.084) of nonfluorosed group respectively. The percentage of calcium and phosphorous (p= 0.02, 0.04) was significantly more in nonfluorosed than fluorosed respectively. The percentage of carbonate (p= 0.001) was significantly higher in fluorosed group than nonfluorosed.

Table I: Mineral content of nonfluorosed and fluorosed cortical bone.

	Nonfluorosed	Fluorosed	t test	p value
fluoride	0.006 ± 0.018	0.520 ± 0.086	1.640	0.118 (NS)
Calcium	30.44 ± 10.00	22.56 ± 6.88	-2.051	0.055 (S)
phosphorous	10.43 ± 4.48	7.463 ± 2.264	-1.871	0.078 (NS)
magnesium	0.357 ± 0.153	0.483 ± 0.259	1.322	0.203 (NS)
sodium	0.103 ± 0.124	0.104 ± 0.134	0.017	0.986 (NS)
zinc	2.52 ± 0.942	2.905 ± 0.731	1.018	0.322 (NS)
potassium	0.546 ± 0.213	0.549 ± 0.237	0.030	0.977 (NS)
carbonate	65.27 + 6.24	53.62 +13.31	- 0.25	0.022 (S)

*** p value calculated using unpaired t test (NS) = Non significant; (S) = Significant**

Table II: Mineral content of nonfluorosed and fluorosed medullary (cancellous) bone.

	Nonfluorosed	Fluorosed	t test	p value
fluoride	0.083 ± 0.084	0.084 ± 0.089	0.026	0.980 (NS)
Calcium	35.67 ± 7.466	27.29 ± 8.00	-2.423	0.026 (S)
phosphorous	12.86 ± 4.10	9.55 ± 2.666	-2.145	0.046 (S)
magnesium	0.238 ± 0.146	0.263 ± 0.195	0.323	0.750 (NS)
sodium	0.105 ± 0.110	0.106 ± 0.122	0.019	0.985 (NS)
zinc	2.288 ± 0.945	2.32 ± 0.711	0.110	0.914 (NS)
potassium	0.646 ± 0.266	0.608 ± 0.426	0.239	0.814 (NS)
carbonate	28.98 + 8.00	47.28 +10.52	4.378	0.001 (HS)

*** p value calculated using unpaired t test (NS) = Non significant; (S) = Significant**

Table legend

Sl. No.	Tables	Title
1	1	Mineral content of nonfluorosed and fluorosed cortical bone
2	2	Mineral content of nonfluorosed and fluorosed medullary (cancellous) bone

DISCUSSION

In our study, A total of 24 human bones (femur) samples (fluorosed and non fluorosed) were collected from orthopaedic section. However, various authors have conducted studies using femur, tibia, fibula, calvaria, rib, vertebra (F. J. Mcclure et al 1958), iliac crest, sternum (I. Zipkin et al 1960), mandible of human bones, rabbits(Sharma K. Susheela A. K. 1988), rats with the sample size varying from 2(Fonseca AA et al 2008), 3 to 5(Jha Mohan, Susheela A.K. 1984),14 (A K. Susheela, Mohan Jha. 1981), 69 (I. Zipkin et al 1960), 127(R. A. Call et al 1965). CF Hildebolt in 1997, in a clinical study reported that there exists an association between the bone densities of jaws and metacarpals, forearm bones, vertebrae and femur. Hence, the femoral bone was selected in the current study.

The percentage of calcium, phosphorous in cortical bone was more in nonfluorosed group; the percentage of sodium, magnesium, zinc, potassium, fluoride, carbonate was more in fluorosed group. (Table 1) The percentage of calcium, phosphorous, potassium, carbonate in cancellous bone was more in nonfluorosed group. The percentage of sodium, magnesium, zinc, fluoride was more in fluorosed group. (Table 2).

As per the authors knowledge this study presents the difference in mineral content of nonfluorosed and fluorosed cortical and cancellous bone for the first time in literature.

Zipkin in 1960 conducted a study to determine the fluoride deposition in human bones after prolonged ingestion of fluoride in drinking water who had drunk water containing 0.1 to 4.0 ppm fluoride. It was concluded that these bones showed no significant histological changes and contained up to 0.548 percent fluoride on a dry, fat-free basis and 1.080 percent fluoride on an ash basis. (I. Zipkin et al 1960).

Analytic chemical studies were conducted by Mcclure in 1958 to determine the effect of a prolonged exposure to drinking water containinig 8.0 ppm of fluoride on the chemistry of human bones. The percentage of fluoride, calcium, phosphorous, magnesium, carbon dioxide at 8 ppm of fluorde level was 0.55%, 26.71%, 11.71%, 6.35%, 3.95% respectively. (F. J. Mcclure et al 1958).

Zipkin in 1960 conducted a study to analyze the effects of water borne fluoride on 69 bone samples. The percentage of calcium and phosphorus in the dry, fat-free bones was normal although they contained as much as 0.4% fluoride; the calcium: phosphorus ratio remained unaffected by fluoride. With increased levels of fluoride in the ash there was a slight increase in magnesium and a decrease in carbon dioxide; the citrate content decreased, a slight decrease in sodium and little or no change in potassium were noted.

Excerpts from studies on mineral content of hard tissues are presented here.

a. The increase in Ca/P ratio observed by electron microprobe x-ray analysis of periosteal and endosteal surfaces, and in the Ca/P ratio observed by chemical analysis of whole bone powder, as an accompaniment to the increased fluoride concentration, is in agreement with previous studies. (West V.C 1971, West V.C 1972) The increased Ca/P ratio can be explained by the fact that high fluoride concentration favours the transformation of amorphous calcium phosphate, which has a low Ca/P ratio, into crystalline apatite, which has a high ratio. (West V.C 1971) Increase in crystal size may also be associated with an increased Ca/P ratio.(E. D. Eanes et al 1965).

b. Although the enhanced Ca/P ratio indicate hypermineralization of the matrix, the results obtained from SEM studies suggest hypomineralization of the collagen fibers in animals which have ingested fluoride. This observed hypomineralization could be explained on the basis of the fact that the OH- position in the crystal lattice is filled by Fluorideion, with an equivalent amount of P03 ion escaping to balance the charge.(Amberg C.H et al 1974).

On the other hand, the mere presence of calcium fluoride is likely to cause the abnormally high Ca/P ratio found in " fluoride-treated bone", although the presence and the exact nature of the compound has not been established either by x- ray diffraction or by electron diffraction studies. It could however, exist as a calcium-rich organic complex with proteoglycans and glycosaminoglycans which have been shown to increase in the bone of fluoride-treated animals. Such a complex may be the explanation for the increase in Ca/P ratio found in their investigation. (Jha Mohan, Susheela A.K. 1984).

Excessive ingestion of fluoride results in the accumulation of fluoride in calcified tissues. Fluoride accumulation in cancellous bone, which has a very high surface/mass ratio is much greater than that in cortical bone.(Susheela A.K, Jha Mohan 1981) From the results obtained it was obvious that fluoride ingestion in excess adversely affects the hydroxyproline content both in cortical and cancellous bones which suggests reduced biosynthesis. Decreased uptake C^{14} proline of in fluoride toxicity both in vitro and in vivo (Susheela A. K. Mukherjee, D. 1981) has also been reported, providing conclusive evidence that the bone matrix of both cortical and cancellous bones is laid down with inadequate collagen.

Other complex mechanisms possibly brought about by high fluoride levels is discussed here. The reduced nitrogen content in cortical bone is possibly due to the reduction in protein biosynthesis and/or collagen biosynthesis. (Susheela A. K. Mukherjee, D. 1981, Proffit W.R, Ackerman J.L 1964) However the unaltered nitrogen content of cancellous bone may be due to increased non collagenous proteins viz proteoglycans, known to occur due to fluoride ingestion. The cancellous bone shows increased zinc, calcium and magnesium contents following fluoride ingestion. Increased zinc content in cancellous bone may be an index of increased metabolic activity (Hammernt S, Mclean F.C.1966) which is evident from increased osteoblastic activity. (Jowsey J 1972) The decreased molar ratio of Mg/Ca indicates that the rate of conversion of amorphous calcium phosphate (ACP) to crystalline hydroxyapatite (HA) has been enhanced. Our observations in this regard correspond with the results reported by Baud and his collaborators.(Baud C.A 1976) However there is considerable variation in calcium and magnesium contents of cortical bone with different phases of fluoride ingestion and this reveals a fundamental aspect of the physiological status of cortical bone. From the above discussion, it is evident that fluoride poisoning produces significant changes in both organic and inorganic constituents of bone. However, the effects of fluoride on cortical and cancellous bone are different. The differential response is due to the intrinsic variations in biochemical characteristics of bone.

As mentioned in Zipkin et al (1960) article, studies done by Wolff and Kerr (1938), Glock, lo Water and Murray (1941) and Call and Greenwood (1958) have found no change in mineral content **(c**alcium, phosphorus or carbon dioxide content of the bones) of human bones by Fluoride after excessive F ingestion. However, Mcclure, Mccann and Leone (1958) reported an increase in carbon dioxide, a reduction in magnesium and phosphorus but no change in the calcium content of the ash of a number of bones of one individual containing up to 0.97% fluoride.

It was confirmed from other in vitro findings that fluoride replaces carbon dioxide(Neuman, W. F. et. Al. 1950), inhibits citrate uptake (Leon singer and W. D. Armstrong 1963), and has no influence on calcium or phosphorus exchange. (Neuman, W. F. et al 1950) An explanation for this result may reside in the fact that sodium, potassium, magnesium, carbon dioxide and citrate are presumed to be confined to the surfaces of the apatite bone crystals, and although in these regards there may be some uncertainty concerning the position of magnesium in the crystal. As regards magnesium, the apparent increase which accompanies fluoride deposition may be explained as due to its affinity for fluoride. (I. Zipkin et al 1960).

The Fluoride water level and Fluoride concentration has been studied. At F level of 1ppm, no difference was found in the Fluoride concentration of various bones. From this extensive survey on the fluoride content of human skeletal tissues, it appears that the deposition of fluoride is directly related to the fluoride content of the drinking water up to 4.0 ppm. The deposition of fluoride in dentin and enamel is also elevated proportionately with an increasing concentration of fluoride in the drinking water.(Zipkin et al 1958).

Although there are a number of reports on the chemical composition of mammalian cortical bone there is a paucity of information on the chemical composition of cancellous bone.[9] From the results it is evident that cortical bone contains more inorganic and less organic and water content than cancellous bone. Density of cortical bone exceeds, has less hydroxyproline, nitrogen, and hexosamines than cancellous bone. Among inorganic constituents analysed calcium, magnesium levels are high in cortical bone. However, zinc, fluoride and phosphorous contents are not significantly different between cortical and cancellous bone. (A K. Susheela, Mohan Jha 1981).

In conclusion it may be added that the cortical bone differ biochemically from cancellous bones with respect to number of parameters that have been investigated. It is therefore unlikely that the response of cortical and cancellous bone to various pathological conditions will be same. (A K. Susheela, Mohan Jha 1981).

Fluoride effect on bone mineralisation is suggested -It is reasonably well established that fluoride poisoning affects the structure and normal functioning of both osseous and non-osseous tissues.(A. L. Ogilivie 1983) The mineralization process in skeletal tissues has been reported to be defective in fluoride poisoning. (C. A, Baud et al 1970) One of the possible explanations for defective mineralization in bone is that the organic matrix of bone might be abnormal. Indirect evidence obtained through autoradiographic (using S35) and other histochemical studies suggest changes in GAG in bone and tooth during mineralisation.(A. L. Ogilivie 1983) The present investigation was undertaken with the objective of examining the effect of fluoride on cortical and cancellous bones with reference to GAG content. And their chemical composition.

Fluoride content in calcified (osseous) tissues: Cancellous and cortical bone has been studied separately for their affinity for fluoride. The data obtained for fluoride content of two different types of bones indicate a significant difference in their affinity for fluoride. The results clearly indicate that cancellous bone has the highest affinity for fluoride and accumulated large amounts of fluoride compared to cortical bone. (Susheela A.K et al 1983)
The studies related to the objectives of our current study are not comparable directly as their subjects, age, sex, water fluoride exposure, methodology vary and differ from this study. The

pertinent studies are as early as 1950s and there is paucity of studies in an active area of human research till date. This could be owing to the nature of fluoride induced diseases which are chronic in nature and seen in later ages as the cumulative effect. As said, there is no cure for this disease and prevention of occurring fluorosis effects is the ultimate treatment.

The government policies should be made compulsory for defluoridation measures and early detection and treatment as the fluoride induced certain changes are reversible.

The overall characteristic change in the organic and inorganic constituents of bone brought about by high water fluoride levels that mineral changes indicates the susceptibility of alveolar bone to periodontal disease too far behind to reason out increased periodontal disease in fluorosed subjects. Further biochemical studies are serum GAG levels and histochemistry of fluorosis affected cementum and bone would throw a conclusive report on fluorosed induced bone loss that is periodontitis.

REFERENCES

1. A.K. Susheela and M Jha. Effect of Fluoride Ingestion On Cortical And Cancellous Bone Composition. IRCS Medical science. Library compendium, 1981; 9(11): 1021-1022.
2. A K. Susheela, Mohan Jha. Biochemical characteristics of cortical and cancellous bone. IRCS Medical Science: Anatomy and Human Biology; Biochemistry; Cell and Membrane Biology; Connective Tissue, Skin and Bone; Experimental Animals; Pathology, 1981; 9: 640.
3. Amberg C.H, Luk H.C, Wag stoff P.K. The fluoridation of nonstoichiometric hydroxyapatite. An infrared study. Can. J.Chem., 1974; 52: 4001.
4. A. L. Ogilivie. J Dent Res., 1983; 32: 386.
5. Baud C.A., Ponezot, J.A., Tochan-Danghy, H. J. Cal. Tissue Res., 1976; 452.(Suppl.).
6. C. A, Baud and S. Bang, in: Fluoride in Medicine, p. 27. Ed, T, L. Vischer. Huber, Bern, Stuttgart, Vienna, 1970.
7. E. D. Eanes, I. Zipkin, R. A. Harper, A. S. Posner. Small-angle x-ray diffraction analysis of the effect of fluoride on human bone apatite. Arch Oral Biol., 1965; 10: 161-173.
8. Fonseca AA, Cherubini K, Veeck EB, Ladeira RS, Carapeto LP. Effect of 10% formalin on radiographic optical density of bone specimens. Dentomaxillofac Radiol, 2008; 37(3): 137-41.
9. F. J. Mcclure, H. G. Mccann, N. C. Leone. Excessive Fluoride in Water And Bone Chemistry comparision of 2 cases. Public health reports, 1958; 73(8): 741-746.

10. Glock, G. E., Lowater, F, Murray, M. N. The retention and elimination of fluorine in bones. Biochem J, 1941; 36: 1235-1239.
11. Hammernt S, Mclean F.C. In Zinc Metabolism. C.G. Thomas, Springfield., 1966; 14.
12. I. Zipkin, F. J. Mcclure and W. A. Lee. relation of the fluoride content of human bone to its chemical composition. Arch oral Biol., 1960; 2: 190-195.
13. Jowsey J. Am. Med,. 1972; 53: 151.
14. Jha Mohan, Susheela A.K. Scanning electron microscopic and electron microprobe x·ray analysis of cortical bone of fluoride treated rabbits. Int J Tiss Reac, 1984; 6(3): 255-261.
15. K L Vandana. Fluorosis and periodontium: A report of our institutional studies. Journal of ICDRO, 2014; 6(1): 7-15.
16. Leon singer and W. D. Armstrong. Comparison of Fluoride Contents of Human Dental and Skeletal Tissues J Dent Res., 1962; 41(1): 23-28.
17. Neuman, W. F., Neuman M. W, Main E. R, O'Leary J., Smith F. A. The surface chemistry of bones-II. Fluoride deposition. J Biol Chem., 1950; 187: 655-661.
18. O. Fejerskov, M J Larsen, A Richards, V Baelum. Dental Tissue Effects of Flouride. Adv Dent Rest, 1994; 8(1): 15-31.
19. Proffit W.R, Ackerman J.L. Science., 1964; 145: 932.
20. R. A. Call, A. Greenwood, W. H. Lecheminant, J. L. Shupe, H. M. Nielsen, L. E Olson, R. E. Lamborn, F. L. Mangelson, R. V. Davis. Histological and chemical studies in man on effects of fluoride. Public health reports, 1965; 80(6): 529-538.
21. Sharma K, Susheela A. K. Effect of fluoride on molecular weight, charge density and age related changes in the sulphated isomers of glycosaminoglycans of the rabbit cancelllous bone. Int J Reac, 1988; 10(5): 327-334.
22. Susheela A.K, Jha Mohan. Experientia, in press, 1981.
23. Susheela A. K. Mukherjee, D. Toxicol Eur Res., 1981; 3: 99.
24. Susheela A.K. Jha M, Koacher, J, Jain S.K. Fluoride and calcified tissues. Fluorosis research workshop proceedings, Kenya, 1983.
25. West V.C. Observations on phase transformations of a precipitated calcium phosphate. Calcil Tissue Res., 1971; 1: 212.
26. West V.C. The in vivo effect of fluoride and tetra·cyclineon phase transformation of a calcium; phosphate. Calcif Tissue Res., 1972; 9: 207.
27. Wolff W. A., Kerr E. G. The composition of human bone in chronic fluoride poisoning. Amer J med Sci., 1938; 195: 493-497.

28. Zipkin, F. J. Mcclure, N. C. Leone, and W. A. Lee. Fluoride deposition in human bones after prolonged ingestion of fluoride in drinking water. Public Health Reports, 1958; 73(8): 732- 740.