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MICROHARDNESS 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 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 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 microhardness of fluorosed and nonfluorosed femoral bone. **Materials and Methods:** A total of 22 healthy nonfluorosed and fluorosed bone (femur) specimens were collected to assess and compare the microhardness (Vickers hardness tester) of fluorosed versus non fluorosed bone. 2 fluorosed specimens were not subjected for the analysis due to required size for measurements of 3 parameters was not adequate. **Results:** The results of the study showed that the mean hardness of the fluorosed bone (222.4 ±4.24) was lower compared to non fluorosed bone (294.4±49.36) and was highly statistically significant. **Conclusion:** The observed changes in microhardness would influence the pathogenesis of periodontal disease and/or outcome of periodontal treatment. Dental fluorosis may soon be designated as environmental risk factor in endemic fluorosed area. Clinicians have to pay attention to treatment of fluorosed and nonfluorosed roots.

KEYWORDS: Dental fluorosis, periodontitis, microhardness, femoral bone.

INTRODUCTION

Fluorosis is a global problem was reported by Dean (Dean H. D. 1993**)**, Lyth (Lyth O 1946) and Murray et. al. (Murray, M.M., Wilson, D.C. 1948) it was first reported from India by Shortt et al (Shortt et. al 1937). Since then there have been a number of reports from different parts of the country. However, fluorosis persists till recent times, as a disease without treatment and cure.

Although, fluorosis was detected, diagnosed and recognized as a well-defined clinical entity as early as 1937 precious little has been offered in terms of amelioration to millions of the Indian people who are crippled and leading in terms of amelioration to millions of the Indian Union. However, the clinical description, radiological findings and other manifestations described by several others from India are still considered as monumental contributions (Sharma K and Susheela A. K. 1988).

Before an effective treatment is established, it is pertinent to take into account the non-skeletal tissue involvement in the disease process. It has been stated that fluorine, one of the most reactive elements is found (as fluoride) in many organs and tissues besides the bones and the teeth (Waldbott G. L. 1973). It is of importance to investigate the mechanism of action of fluoride ions and the degree of involvement of various other body tissues especially those which do not possess a buffering agent like apatite crystals which are believed to neutralize fluoride ions in bones, before the defluoridation agents like serpentine and magnesite could be employed effectively.

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, 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 of literature dealing with fluorosis effect on biological tissues like bone and cementum. (K L Vandana 2014)

The histologic and hardness studies are minimal as compared to mineral studies. There is great amount of

literature added by A.K Susheela and her research team (Susheela A.K and Jha M.1981; Susheela A.K and Jha M. 1980; Jha M and Susheela A.K.1984) by artificially feeding rabbits with high dose of sodium fluoride. The short coming is lack of control group (nonfluorosed) in many of their studies. As of now there are no human studies comparing bone from nonfluorosed and fluorosed areas. As per the authors knowledge, the current study is the first one to attempt considering the following objective: To assess and compare the hardness of fluorosed versus nonfluorosed bone.

Clinical Relevance

Scientific rationale for the study: It has been explored in a review that an epidemiological association exists between fluorosis and periodontal disease. There is a scarcity in literature dealing with effect of fluorosis (changes in microhardness) on biological tissues like bone.

Principle findings: mean hardness of the fluorosed bone was lower compared to non fluorosed bone and was highly statistically significant.

Practical implications: The observed changes in microhardnes of fluorosed bone would influence the pathogenesis of periodontal disease and/or outcome of periodontal treatment. Dental fluorosis may soon be designated as environmental risk factor in endemic fluorosed area. Clinicians have to pay attention to treatment of fluorosed and nonfluorosed roots i.e, periodontal therapy (during scaling and root planning), endodontic treatment, and orthodontic treatment (alteration of orthodontic forces).

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 were 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 microhardness (Mechanical Property) of cortical bone

For the evaluation of microhardness, each bone section embedded in acrylic were positioned in an adjustable Vickers hardness tester. The clamp was locked into a position so that the exposed surface stayed parallel to the horizontal plane, immobilizing the specimen completely during the measurement, where Vickers micro hardness measurements were taken at room temperature. Penetrations on cortical bone were carried out with the Vickers instrument. Care was taken in order to avoid the possible interference of one penetration being too close to the other. Using a light microscope coupled to the microhardness tester each of the markings made with the spherical drill was located (Fabiano Ribeiro Cirano et al 2004). Next, maintaining the alignment with these markings, penetrations on cortical bone were carried out with the Vickers instrument. The penetration diagonals were measured. and through these values, the microhardness of that spot was calculated. For this experiment, the microhardness tester was calibrated at 30 kg applied for 15 seconds.

The microhardness of cancellous bone was not attempted due to technical difficulties.

Statistical analysis

The data obtained from hardness assessment was entered and data was compiled on MS-excel sheet. It 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 hardness of fluorosed versus non fluorosed bone.The results of the study are interpreted in table .1.

The results showed that the hardness values varied between 247 VHN and 406 VHN for the healthy non fluorosed bone sections and, for the fluorosed bone sections, the values ranged from 218 VHN to 227 VHN. The mean hardness of the fluorosed bone (222.4 ± 4.24)

was lower compared to non fluorosed bone (294.4 ± 49.36) and was highly statistically significant

Table 1: Microhardness of nonfluorosed and fluorosed cortical bone.

Cortical bone	Mean ± S.D	P value*
Nonfluorosed	294.4 ± 49.36	p = 0.000 (HS) t = - 4.5
Fluorosed	222.4 ± 4.24	

***p value calculated using unpaired t test (S) = Highly significant**

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 1951), 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 (F. J. Mcclure et al 1958), 3to 5 (Jha M., Susheela A.K, 1981), 14 (Susheela A.K, Jha M 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. (CF Hildebolt 1997). Hence, the femoral bone was selected in the current study.

In the current study, In nonfluorosed cortical bone, hardness was statistically highly significant (294.4±49.36, p = 0.000) as compared to fluorosed bone (222.4 ±4.24). A study was conducted by Chandler et al in 2011 to measure the mechanical properties of individual osteons of dog's femur bone using nanoindenter. Bone samples were obtained from femur of skeletally mature male dogs. 2 mid femoral cross sections of 3 mm thick were made and polished. These sections were transferred to custom made polycarbonate specimen holder and identification and mapping of the labeled and unlabeled osteons under an epifluorescent microscope (Olympus BX 51, Tokyo, Japan) was done. The indenter was pressed into contact with the test material at a rate of 10nm/sec to a peak depth of 500nm for 30 seconds and then withdrawn. It was concluded that new and old osteons have significantly different mechanical properties and that those properties are directly related to the degree of mineralization (Chandler, AZ .and Sarandeep Huja 2011).

Difference in F and NF bone can be discussed as follows: The difference in mechanical properties is directly related to the degree of mineralization has been suggested. The possible reason could be that mineralized osteons will be more resistant to permanent deformation than newer osteons. This finding is consistent with the observation that excessive remodeling, stimulated by micro-cracks, increases the likelihood of stress fracture, and that phenomena that result in poor mineralization tend to make bone more vulnerable to excessive deformation and fracture (osteomalacia). Yet, the characterization of bone should not be over-simplified. As a composite, it is likely that the mechanical properties of compact bone are determined by complex interactions between osteons of differing properties, not simply by a volume-weighted average (Chandler AZ and Sarandeep Huja 2011).

Newly formed osteons in femur had lower modulus (34%) and hardness (41%) than older osteons found in femoral cross sections. These data provide information on the indentation moduli of osteons during an early phase of mineralization compared to osteons that have completed mineralization (Huja S .S et al 2006).

Hardness measurement of biologic material is method dependent their findings were in agreement with another indentation study of osteonal bone, in which authors found that the dominant time-dependent deformation mechanism for bone was viscoplasticity (Huja S.S et al 2010).

Future work should focus on achieving a better understanding of this tendency. However, because bone tissue at this scale does exhibit viscoplasticity, hardness measurements will be sensitive to test method. Hardness measurements should be compared only if they have been determined by the same method. There is a need to compare the hardness of supporting alveolar bone in both health and disease.

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.

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CONCLUSION

The microhardness of fluorosed bone is lower (222.4 ±4.24) than nonfluorosed bone (294.4±49.36). The

observed changes in microhardness thickness of fluorosed bone would influence the pathogenesis of periodontal disease and /or outcome of periodontal treatment.This may the reason of higher occurrence of periodontitis as shown in the study by K. L. Vandana .Dental fluorosis may soon be designated as environmental risk factor in endemic fluorosed area. Clinicians have to pay attention to treatment of fluorosed and nonfluorosed roots i.e, periodontal therapy (during scaling and root planning), endodontic treatment (during root canal treatment) and orthodontic treatment (alteration of orthodontic forces). The possible shortcoming of this self-funded pilot study is the limited number of specimens Further studies can be done using nanoindentation method, histologic and mineral content of bone.

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