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Original Article

An In Vitro Study of Histology of Nonfluorosed and Fluorosed Bone

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ABSTRACT

The study of the epidemiologic association between fluorosis and periodontitis, as well as the effects of fluorosis on periodontal tissues and the effects of periodontal therapy on teeth of non-fluorosed and fluorosed. There has been limited research into the effects of fluorosis on organic tissues for example bones. The biomechanical, histology and biochemical properties of fluorosed bones have still not been studied in alveolar bone, which, like extremities, is an important component of the periodontal tissue. **Objective:** The goal of this research was to compare the histology of fluorosed femur bones to that of non-fluorosed femur bones. **Method:** 40 non-fluorosed and fluorosed healthy bone (femoral) samples were tested using a microscope to compare and assess the histology of non-fluorosed with fluorosed bone. **Results:** When comparing the non-fluorosed group (11.835.21, 9.853.45) to the fluorosed group (7.724.42, 6.702.42), bone of cortical and cancellous cellularity was shown to be substantial in term of statistics. The non-fluorosed and fluorosed bone had the same trabecular density statistically not significant, p-value is equal to 0.726]. Nonfluorosed bone had thick trabeculae, while fluorosed bone had short and thin trabeculae. **Conclusion:** The identified histologic variations could affect the pathogenesis of periodontitis / the efficacy of periodontal therapy. In widespread fluorosed regions, dental fluoride could likely be identified as an important public health issue.

INTRODUCTION

Fluorine is indeed a key denominator in the crust of the earth and is required for bone and tooth calcification. Fluoride ions have played a key role in significantly lowering caries during the last forty years. Excess fluoride exposure can disrupt the homeostasis of bone, the development of enamel (enamel fluorosis), and calcification Fluorosis severity on periodontium soft and hard tissues is dosage dependent, as well as the exposure of fluoride time and period throughout growth [1]. While "fluorosis" is a national and global issue, few studies are examining the effect of fluoride on "periodontal tissues", which are likely known to be periodontitis due to changes including both soft and hard tissues of the periodontal tissues [2]. While developing a successful treatment, it is necessary to consider the involvement of non-skeletal tissue in the disease process. Fluoride ions, among the most volatile components, have been detected such as fluoride in several tissues and organs other than the teeth and bones [3]. Before defluorination substances such as serpentine and magnesite can be used effectively, it is critical to study the of fluoride ions mechanism and the intensity of multiple numerous different tissues, particularly those that lack a buffering agent such as crystallites, which are used to neutralization of fluoride ions into the bones. In contrast to periodontal tissues, the research on the effect of fluoride on dental caries is thoroughly addressed. "Moreover, 15th

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years of scientific research and a recent review have addressed not only an epidemiologic study linked between fluoride and disease of periodontal then it also the impact of structures of fluorosis in periodontal, as well as a comparison of the impact of treatment of periodontal on teeth which is fluorosed and non-fluorosed" There is a limited information that tells us the impact of fluorosis on human body tissues for example cementum and bone[4].
The histological components of fluorosed bone have received limited attention. "A clinical study [biopsied specimen] revealed that root surface of fluorotic is asymmetrical and have deposit a huge calcified masses at the apical portion of the teeth in the form of excessive amounts of fluorine as well as cementosis, osteosclerosis and roots of periapical resorption radiographically" [5]. According to the literature, the bone histology from an endemically fluorosed region has not been researched. "As a result, a new research area of fluorosis is to compare of fluorosed with nonfluorosed bone" The study's purpose is to evaluate differences in the histology of fluorosed and nonfluorosed bone.

METHODS

The orthopedic department of Lady Reading Hospital provided 20 healthy non-fluorosed and fluorosed bone (femoral) specimens. Participants of both genders aged 30-50 years were included. All participants provided written consent and ethical clearance. The bone specimens had to fulfill the basic inclusion criteria includes, Systemically healthy individuals' bone samples, "Following criteria for the selection of the Fluorosed subject was depend who survive in the water fluoride area for 5 to10 years that consume the fluoride water above the levels 1.2 to 3 ppm and the levels of Peshawar water fluoride "0.2 mg/l to 2.41 mg/l" Participants having enamel with mottled appearance, that is, fluorosis stains on the teeth were evaluated using "Jackson's simplified fluorosis index scores C, D, E, and F". Bone samples were collected from participants who had surgical intervention after fractures due to trauma in which a portion of the bones (femur) was removed. The bone samples were chosen using the same criteria as the fluorosed teeth. Participants' metabolic bone problems "thyroidism, Paget's disease, hypophosphatasia, and infectious diseases" were excluded. Non-fluorosed and fluorosis good health bones were taken and placed in a jar containing 10percent neutral buffer paraformaldehyde. paraffin-embedded blocks were prepared. The collected bone samples were decalcified using nitric acid (10%) at 25°C, with changes in solution at periodic times until the endpoint was achieved. For tissue preparation, decalcified bone was washed with water (24 h) before being processing routinely. Following that, the prepared bone was embedded into the wax which is paraffin and masses of tissue were obtained. Segments were cut with a microtome and mounted to a slide. Tissue samples then deparaffinized with xylene about 28 minutes before being rehydrated in a decreasing ethanol sequence (100 percent for 25 minutes, 90 percent for 5 minutes, and 80 percent for 5 minutes) and rinsed in de-ionized- water for 25 minutes. "Section of the rehydrated and deparaffinized were immersed in Hematoxylin for 5 minutes before being it washed with the opened water for the removal of stain in excess mount for hematoxylin and eosin (H&E) staining" The stain section hematoxylin was dipped once in 1 percent alcohol before being immersed in bluing. They were then dipped in eosin for two min, rinsed with water to remove stain in excess amount, dehydrated alcohol sequence in an ascending orders (80%, 1 minute, 90%, 1 minute, and 100%, 1 minute), cleared in xylene, and prior to mounting with "DPX" for histological evaluation. The relevant parameters were analyzed: The density of cells in cortical and trabecular bones was measured at 40X power and averaged across 5 fields, Under 40X magnification, the occurrence or lack of latent and reversing appearances was also seen, The presence/absence of osteoclasts was related to the presence/absence of how ships lacunae, The density of Trabecular was graded as either sparse (+) or packed (++), Bone marrow (fatty or red). The results from the histologic examination were recorded, assembled, and statistically analyzed using SPSS 20. The t-test and Mann – Whitney U were used for the contrast of the groups. The p-value of 0.05 was considered significant.

RESULTS

To examine and compare the histology of fluorosed by non-fluorosed bone, almost 20 healthy non-fluorosed and fluorosed bone (femur) samples were collected. Cellularity of cortical and cancellous bone is statistically significant in the non-fluorosed category (11.835.21, 9.853.45) compared to the fluorosed group (7.724.42, 6.702.42). as shown in Table 1. The density of Trabecular was the identical in non-fluorosed and fluorosed bone (p-value is equal to 0.726). On the other hand "Trabeculae which were thin and short in size present in fluorosed bone and thick in non-fluorosed bone. The lines of resting and resversing were more prominent in non-fluorosed bone than in fluorosed bone". Content of Fatty marrow were observed in equally groups, but in case of osteoclasts presence in all patients in which osteoclasts were found in non-fluorosed bones and few in fluorosed bone (Table 2).

Bone	floursed	Non- flurosed	p-value	t-test
Cortex Cell	11.83 ± 5.21	7.72 ±4.42	0.034 s	-3.57
Cell in cancellous	9.85 ± 3.45	6.70 ±2.42	0.020 s	-3.99

Table 1: Density of cell in Fluorosed and non- Fluorosed bone of

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Cancellous and Corticol calculation of p-value, t-test (s is significant)

2	2
1	1
2	2
1	1
2	2
2	2
2	2
1	1
2	2
2	2
2	2

Table 2: Compares the densities of bones of non-fluorosed and fluorosed Mann-Whitney U test = 52; p=0.726

DISCUSSION

A total of 40 human bone (femoral) specimens (fluorosed and non-fluorosed) were evaluated in our investigation. Many authors have carried out investigations on the femoral, tibial, fibula, calvaria, ribs, vertebrae, iliac crest, and mandible of bones of humans, rabbits, and mice, with sample sizes ranging from 2 to 5, 14 , 69 , 127 . In 1997, study stated in a clinical investigation that there is a link between the bone density of the jaws and the carpal bones, forearms bones, vertebra, and femoral. As a result, the femur bone was chosen for the current research.Decalcification with 5percent nitric was utilized in our investigation. The preservation of living hard tissues is important for knowing sub-cellular structure and function [5]. Mostly in cases of tissue like bone and teeth, cutting tiny amounts manually is difficult. These tissues should be processed to eliminate calcium phosphate through a process called "decalcification," which softens the tissues to be processed by a microtome machine. "Decalcification is accomplished through the use of chemicals, whether acid to make solvent salts or calcium/chelator mediators that adhere to ions of calcium. Decalcification of Microwave is a revolutionary approach that was shown to be faster than the manual procedure [9]. In this literature, there are no comparison studies on the valuation of non-fluorosed and fluorosed bone of corticol and cancellous histologically. The cell density of corticol bone and trabecular marrow, the occurance or lack of resting lines and reversing lines, the existence/ deficiency of Osteoclasts, density of trabecular, and types of marrow were all evaluated in this study (red or yellow) [10]. Cell density in non-fluorosed & fluorosed cortical and cancellous bones Cellularity was substantially higher in non-fluorosed cortical and cancellous bones (11.835.21, 9.853.45; p-value is equal to 0.035, 0.021) than in fluorosed cortical and cancellous bones (6.613.31, 5.691.31), accordingly. Other histological criteria including rest and reverse lines, marrow contents, and osteoclast were examined in the present study. Non-fluorosed bone has more noticeable resting and reversing lines than fluorosed bones. Both groups had high-fat content in their marrow. Non-fluorosed bone participants had osteoclast in abundance, but fluorosed bone patients had few to none. The cause for the preceding observation must be explained. Bone trabecular density these non-fluorosed and fluorosed bone had the identical trabecular density [statistically non-significant, p= [0.726]. Non-fluorosed bone had thick trabeculae, while fluorosed bone had short and thin trabeculae. The literature's clinical and experimental findings accept the concept of an important activity of fluoride [11] on the osteoblast and osteoclast cell populations. This influence is regularly observed, in the early stages of administration [12], and shows functional and morphological cell alterations that indicate greater activity in existent metabolic cells [13]. Fluoride's potential to enhance bone densification is of two types, according to an in vitro and in vivo experimental investigation employing sustained-release bone cement which is sodium fluoride. Biochemically, fluoride quickly binds to the hydroxyapatite mineral structure of bone, forming fluorapatite with enhanced the properties of mechanical and biochemical; and biologically, the ion of fluoride acts directly on osteoblasts, promoting differentiation and resulting in an osteoid wall of bone which is enlarged trabeculae as well as an improved the capacity of trabeculae. Such influence is regularly observed, in the initial steps of administration, and shows itself as functional and morphological cell alterations that indicate greater activity in existent metabolic cells. These benefits, if achieved properly by slow fluoride release from bone cement, might be highly helpful in preventing periprosthetic bone resorption [14,17]. The most notable histo-morphological changes were in the component of trabecular [18], which was replicated in a higher total volume of trabecular in both the portion of mineralized and non-mineralized. Clinical investigators still suspect an excessive amount of non-mineralized tissue, because of osteoid tissue in excess amount may obstruct treatment objectives or potentially raise the risk of long-bone cracks, mainly of the hips [19]. In clinical studies the other finding, appear to support Kanis and Meunier's hypothesis [19] that such changes of histomorphometry are nearly linked to the quantity of fluoride which varies depend on the dose which intake daily and secondly, the treatment time. Additional osteoid tissue is commonly detected in the early phases in oral treatment and at high doses [20]. There have been reports of histologic alterations in fluorosed bone. Bones become radiopaque on X-Ray because of accumulation of huge partially mineralized and less organized bone, which is not attributable to enhanced mineralization/resorption failure [20]. Various publications have also documented high mineralization of cortical bone in widespread and fluorosis

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industrially [10]. This occurrence of elevated glycosaminoglycan concentrations could explain the mineralized collagen fibres detected in the resorbed areas. Sulfated glycolsaminoglycans, which are powerful hindurance of mineralization, have been found in significant amounts in bone [1]. Mineralization of collagen fibers requires the removal of glycosaminoglycans. As a result, excessive glycosaminoglycan concentrations could be the cause of inadequately mineralization collagen fibrils [12]. Fluoride's role in raising GAG, Fluoride has been shown to raise GAG, or dermatan sulfate, levels in cortical bone, which limits development. There has also been evidence of elevated derma tansulphate in human teeth in cases of dental fluorosis [7]. Fewer cross-link precursors of collagen & collagen synthesis could explain the alteration in the shape of fibers of collagen and the medium [16]. As a result, decreased collagen cross-links and synthesis, as well as a considerable rise in Glycosaminoglycan's might be the causes of uncultured alterations forms in fluoride bone. Fluoride accumulation in calcification and non-calcified tissue is associated with a variety of symptoms. This has been investigated in particular with regard to collagen fibers and non-collagenous components. The amount of hydroxyapatite higher in the cancellous bone rather than corticol bone. These results indicate that hydroxyproline contents are lowered in both osseous and non-osseous tissues during fluoride overdose. This could have an impact on the tissue's collagen content. This shows that collagen produced after fluoride consumption would be under oxidized and inter-linked inadequately, and is promptly assimilated. Collagen deposition in osseous and nonosseous tissues is irregular as a result of high fluoride consumption [15]. The nature of the cells in osteoid related to cartilage cells, chondrocytes and formation of osteoid resemble to the fibrocartilage and trabeculae is also present in chondrocytes due to the intoxication of Fluoride. The variation of the striking structural were observed in the corticol bone due to the ingestion of fluoride in huge amount (1) that why the thickness of corticol increased (2) and enhanced the diameter of Osteon [21]. In this study, the density of cellular cortical & cancellous bones was investigated. The study of the effects of fluoride on bone is an excellent source of information for studying biochemical reactions in fluorosed bones.

CONCLUSIONS

The studies that are similar to the goals of our current investigation aren't comparable because their participants, age, gender, water fluoride consumption, and technique differ from our study. The relevant studies date back to 1950, and there is limited research on a prominent aspect of human study currently. This may be due to the chronic situation of fluoride-related disorders that shows up later in life as accumulative effect. As previously said that there is no treatment for this disease and no prevention, the government policies should be mandatory for the measurement of defluoridation, initially detection and treatment as the reversible changes in fluoride induced form.

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