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Evaluation of mandibular alveolar bone & tooth cementuminosteoporotic postmenopausal rat model

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Abstract: Back ground: Osteoporosis that usually occur during postmenopausal stage is considered a progressive bone disorder. It was proposed that the principal cause of osteoporotic postmenopausal isestrogen deficiency. The present research was aimed to examine histologically the status of alveolar bone & tooth cementum in osteoporotic postmenopausal rat model. **Material and methods:** Twenty virgin female rats were split into two equal groups; ovariectomized (OVX) rats, Sham groups. After four weeks, the bone samples containing the molar teeth were collected from the euthanized animals and processed for light microscopic examination. **Results:** Histologic and histomorphometric analysis of alveolar bone samples revealed significant reduction intrabecular bone pattern and disorganized bone structure in OVX rats compared with sham rats. OVX rats showed more osteoclasts than sham rats. Loss of continuity and resorption areas of cementum were noticed in OVX rats. **Conclusion:** OVX induced osteoporosis can alter the microstructural of the tooth cementum and alveolar bone.
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Keywords: Alveolar Bone, cementum, estrogen, ovariectomy, osteoporotic postmenopausal, rat.

1. Introduction

Osteoporosis is a growing public health problem of known by micro-architectural degradation of bone and decreased bone mass, subsequently enhanced the fragility of bone and the risk of fracture increased [(1)]. The most common etiology of osteoporosis in women during menopause is the deficiency in estrogen levels which are responsible only or with factors for poor skeletal growth in old men [(2)].

The major estrogen effect mainly concerned in regulating both bone growth & adult bone turnover and maturation. Estrogen is required throughout bone development to properly close epiphysal growth plates. The lack in estrogen in menopause induces both cortical and cancellous bone loss. Due to microfractures and penetrative resorption, in can cellousone the highly elevated bone resorption contributes to overall bone damage and degradation of local construction. The first withdrawal reaction of estrogen in cortical one is increased endocortical resorption [(3)].

The estrogen role in controlling bone homeostasis and preventing bone loss is known to be significant [(4)]. The estrogen lack creates a bone remodeling imbalance, which allows the suppression of bone formation [(5,6)]. Bone homeostasis control occurs through protein expression balance and pro-inflammatory cytokines [(7,8)], also to discovery of protein receptor activator of nuclear factor-k B ligand (RANKL), proposing the effect on periodontal tissues bone resorption [(9,10)].

In the circumstance of osteoporosis is lead to the periodontal breakdown of tissues because it can intensification resorption of bones and suppress appropriate healing of tissues and eventually increase an extent of the pre-existing periodontal illness. As seen in the osteoporotic body, bone density is decreased which subsequently followed by elevation in the liability to alveolar bone damage and in advanced cases regenerative periodontal processes may occurred [(11-13)].

Recently, most of the experimental studies dealing with osteoporotic animal model were rarely discussed the microstructural changes of tooth cementum beside the quantitative changes of bone measurement. Hence, it is of prime importance to investigate the impacts of deficiency of estrogen on the level of microstructural alterations in alveolar bone of rats and tooth cementum in experimental osteoporotic postmenopausal rat model.

2. Material and Methods

Animals

Twenty virgin female Sprague–Dawley rats (3 months- old and approximately 250-300g in weight) were selected for the study. Animals were served in

animal house of Histology Department, Faculty of Medicne, Tanta University. The rats were kept on a 12 day night / dark at (22 °C, 55%- 70% humidity). They received a diet of standardized pellets and free acess to tap water ad libitum. We have followed the guidelines of the Institutional Committee of Ethic at Faculty of Dentistry, Tanta University.

Experimental design

After one week of adaptation to the new laboratory conditions, rats are randomly allocated to two equal groups: Group I,10 rats underwent OVX operation bilaterally; Group II: 10 rats were subjected tosham operation of OVX.

Bilateral ovariectomies:

Bilateral ovariectomies were conducted with a minimally invasive surgical procedure under sterile conditions. Injection of chloral hydrate intraperitoneal (10%, 4 ml/kg body weight) was used to anesthetizedanimals. In the OVX groups, 10 mm longitudinal bilateral lumbar lateral skin incisions were done. The enterocoelia was then exposed to blunt muscle and dissection of peritoneum. The bilateral ovaries were carefully removed [14,15] (Fig. 1A, B, C, D). The sham-operated groups underwent a similar surgical procedure, exposing the ovaries, and part of fat tissues around the ovaries were removed and replacing ovaries in the same position.

The mandibles of sacrificed animals (with an overdose of pentobarbital sodium) were evaluated after four weeks. Mandibles were dissected and their right & left halves were collected and immediately fixed with 4% buffered formalin solution.

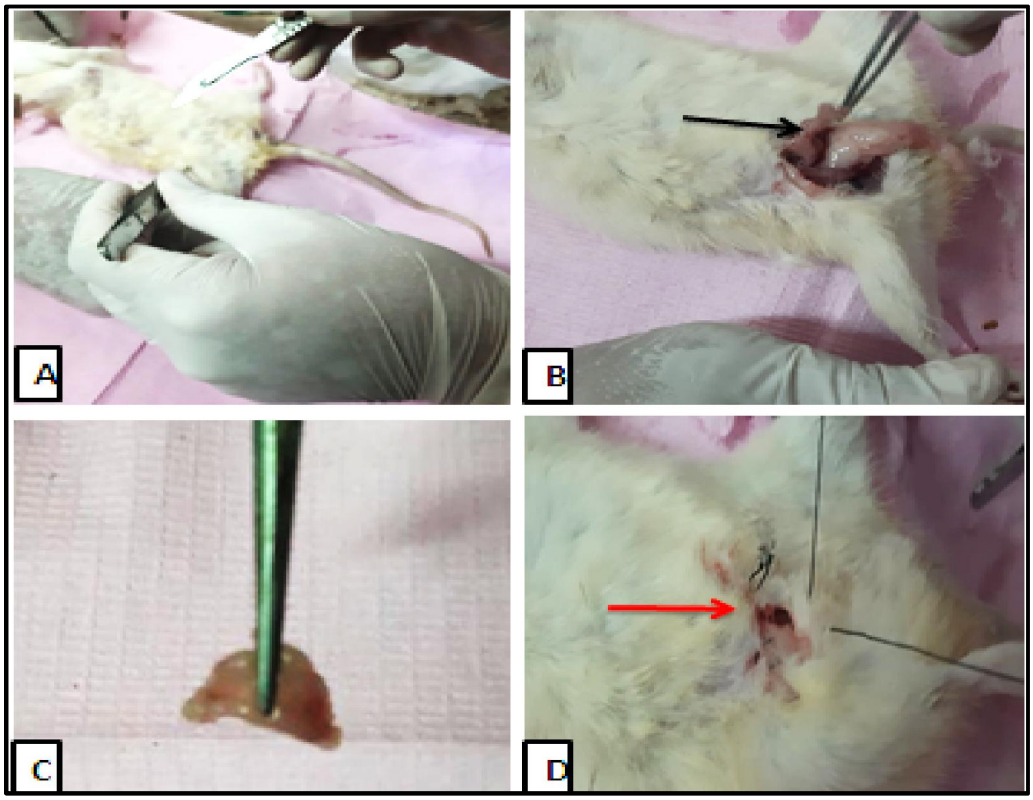

Fig. 1: OVX in rat. (A) Anesthetized rats are put from the back on the operating table. (B) The ovary (black arrow) is exposed, (C) the OVX complete. (D) Stitching of muscle, peritoneal cavity and skin (red arrow).

Histological Examination

After fixation, the specimens were decalcified in 10% EDTA solution, pH 7% (Sigma– Aldrich). After complete decalcification, specimens were washed in distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and used paraffin was for embedding. Serial sections (5µm) were cut. Right mandibular halves were stained with hematoxylin and eosin (H & E), whereas left halves stained by Masson Trichrome (MT). Images of the specimens sections were examined by LM (Leica ICC50 HD) at Faculty of Dentistry, Tanta University.

Statistical evaluation

Histomorphometric analysis of the bone trabeculae:

The slides were processed for massontrichrome stain, then they were visualized in a Leica L.M, DM500 with Leica (ICC50 HD) Camera system. The images of serial sections were analyzed for bone

trabeculaequantitative measurement using the Image J analysis system (Image J 1.48s).

Quantitative analysis of osteoclast cells counting:

Images of the H & E stained sections were analyzed for osteoclast cells counting, 2 blinded observers examined the sections, the cell types were identified by morphological and histological based.

t-test were used to performed all statistical analyses. Means and standard deviations were used as expression of all values. The statistical software "SPSS 20" (Statistical Package for Scientific Studies) were used to carry out all statistical analyses. Results were expressed in the form probability value (p-value) that was differentiated into: very highly significant when p-value ≤ 0.001, highly significant when p-value ≤ 0.01, significant when p-value ≤ 0.05, non-significant when p-value > 0.05.

3. Results

Light microscopic results

1. H & E Staining:

At the end of four weeks, group I (sham group) demonstrateda normal trabecular bone pattern with well-defined medullary spaces. Normal counts of osteocytes was recorded in samples of alveolar bone from sham rats and typical size lacunae of osteocytes (Fig. 2A, B, C). Another section of control group Buccal & lingual specimens of the furcation region through the mesial and distal root of the first molarexhibited well-organized cortical lamellar bone, fibers of periodontal ligament were obliquely oriented (Fig. 3A, B).

At the end of four weeks group II (OVX group) revealed an increase in bone marrow space and the trabecular projections appeared longer and more finger-like forming a lace-like pattern. Compared to the sham group, OVX group showed granulation tissue which, in some areas, replaced by more mature connective tissue (CT) characterized by angiogenesis and inflammatory cells infiltrate. A high amount of osteocytes were detected in alveolar bone obtained from ovarectomized rats (OVX) in comparison with sham rats. Moreover, the lacunae of osteocytes were greater in OVX rats in relation to control (sham operated) rats (Fig. 4A, B, C). Another section of OVX group labial & lingual sections of alveolar bone proper at incisors area showing an apparent indication of corrosion of trabecular configurations with fat-rich bone marrow-like tissue. A reduction of lamellar bone structure in alveolar bone proper was observed in OVX rats. Osteoclasts were recognized along its margin. In the OVX group, loss of continuity and resorption areas of cementum were noticed (Fig. 5A, B, C).

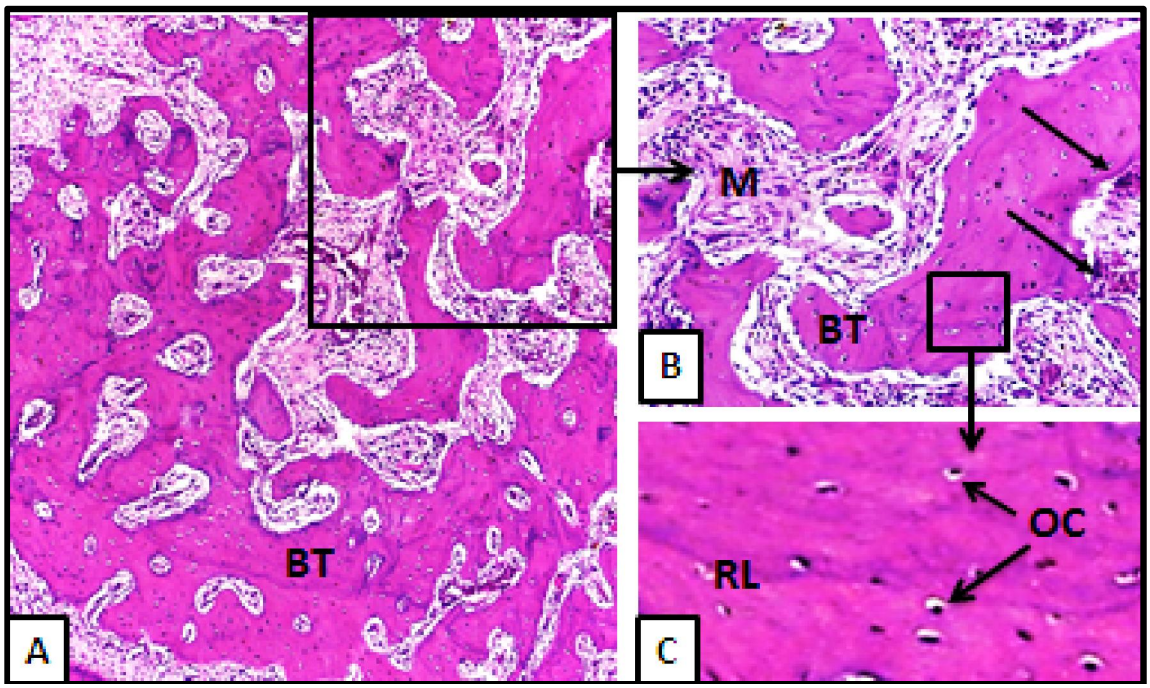

Fig. (2): Photomicrograph of a group I (control group) showed A) healthy lamellar bone structure (H & E, OMX100). B) Higher magnification of (A), the sham group exhibited a normal trabecular bone (BT) pattern with well-defined medullary spaces (M), osteoclasts (arrows) (H & E, OMX200). C) Higher magnification of (B) represented well defined bone trabecullae with regular number and size osteocytes (OC) and reversal lines (H & E, OMX400).

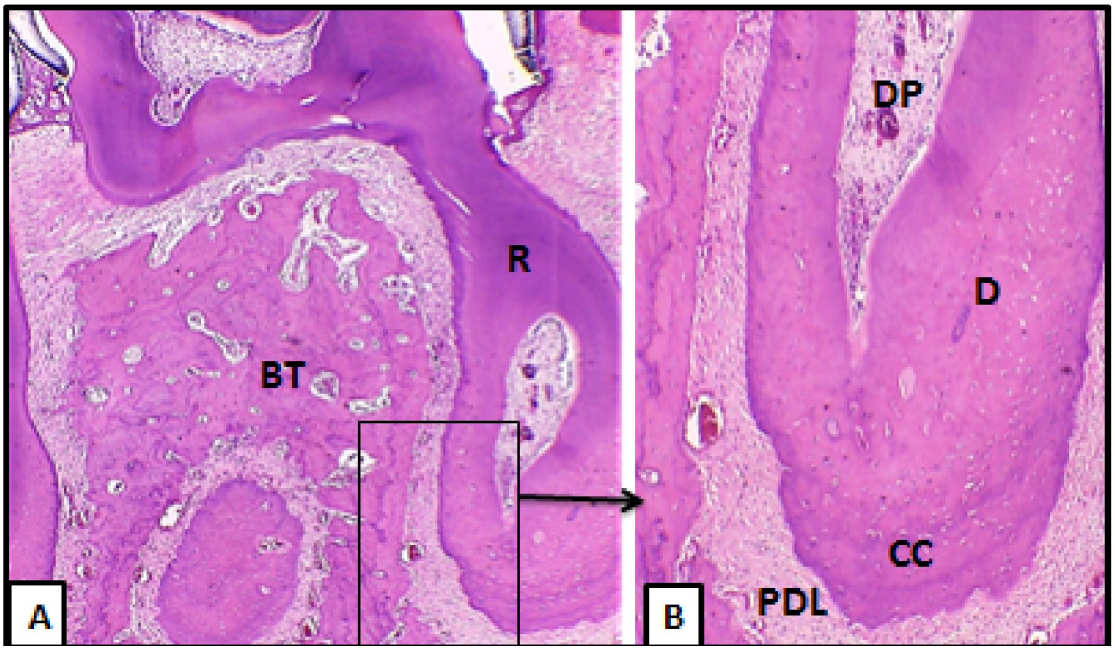

Fig. (3): Another section of the control group, A) Buccal & lingual sections of the furcation area between the mesial root and distal root of the mandibular first molar showed normal volumes of the alveolar bone trabeculae (BT) in the inter-radicular area, root (R) (H & E, OMX100). B) Higher magnification of (A) showed regular cellular cementum (CC), dentin (D) and pulp tissue (DP) and obliquely oriented periodontal ligament (PDL) were noticed (H & E, OMX200).

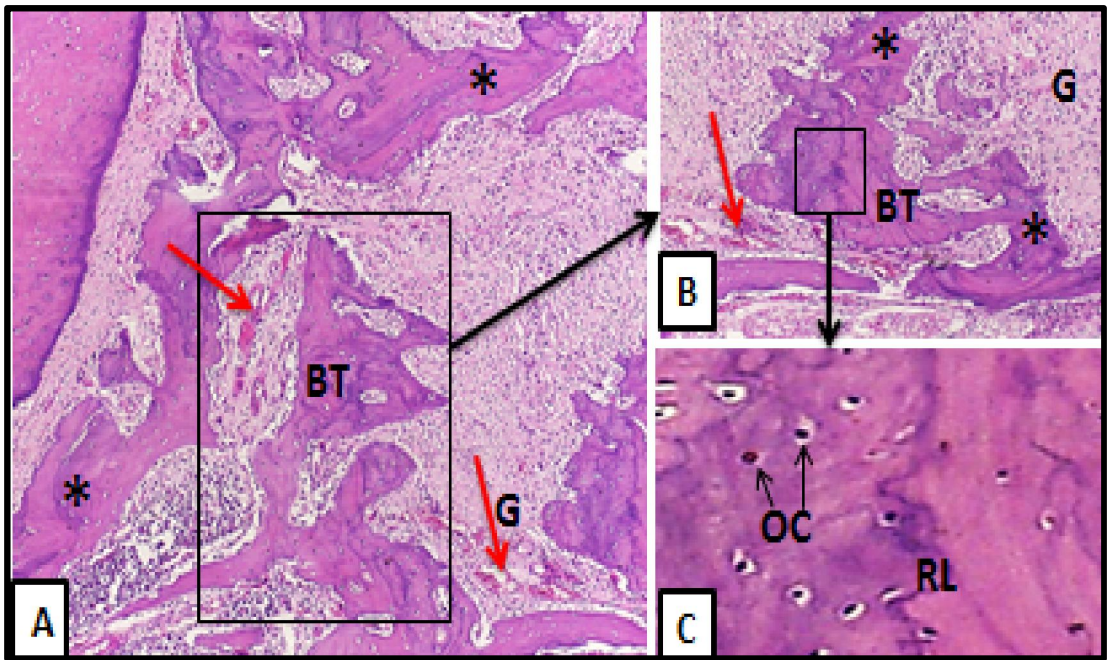

Fig. (4): Photomicrograph of a group II (OVX group) A) showed smaller lamellar bone structure (BT), an increase in number of finger-like trabecular projections (*) (H & E, OMX200). B) Higher magnification of (A) revealed a powerful change in the trabecular bone pattern, granulation tissue (G), angiogenesis (red arrow) (H & E, OMX200). C) Higher magnification of (B) showed. A greater number & size of osteocytes. Reversal lines (RL) (H & E, OMX400).

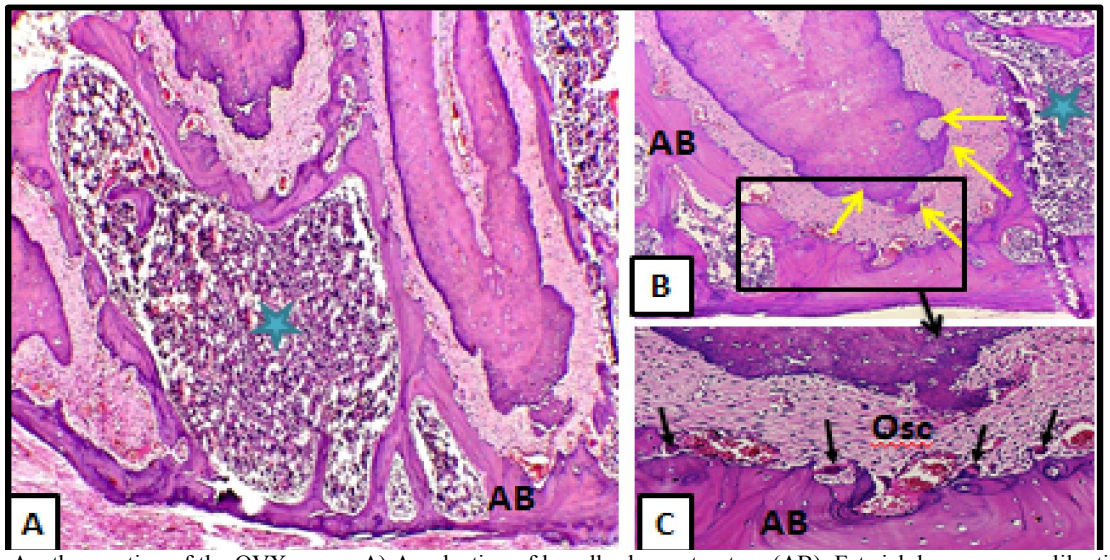

Fig. (5): Another section of the OVX group, A) A reduction of lamellar bone structure (AB). Fat-rich bone marrow-like tissue () (H & E, OMX100). B) OVX group displayed fatty degeneration marrow spaces. Loss of continuity of cementum (yellow arrows), and resorption areas of cementum were noticed (H & E, OMX100). C) Higher magnification of (B) A significant increase in osteoclasts (Osc). (H & E, OMX200).

2. Masson's Trichrome staining:

At the end of four weeks, group I revealed typical compound of collagen in the osteogenic zone, specifically collagen and reddish-colored matured bone (Fig. 6A, B).

At the end of four weeks, group II (OVX group) revealed small thin mature bonetrabeculae. Bone tissue formation is observed in small areas, while other areas showed granulation tissues with Inflammatory cells infiltrate (Fig. 7A, B).

3. Statistical quantitative evaluation of the bone trabeculae and osteoclast cells counting:

The greatest mean value of bone trabeculaequantitative measurement was recorded in group I with the least value obtained in group II. While, the greatest mean value of osteoclast cells counting was recorded in group II with the least value obtained in group I. T-test analyses revealed a highly significant difference between the two groups in case of bone trabeculaequantitative measurement and osteoclast cells counting (p-value 0.000> and p-value 0.001> respectively) (tab.1 & 2) & (fig.8).

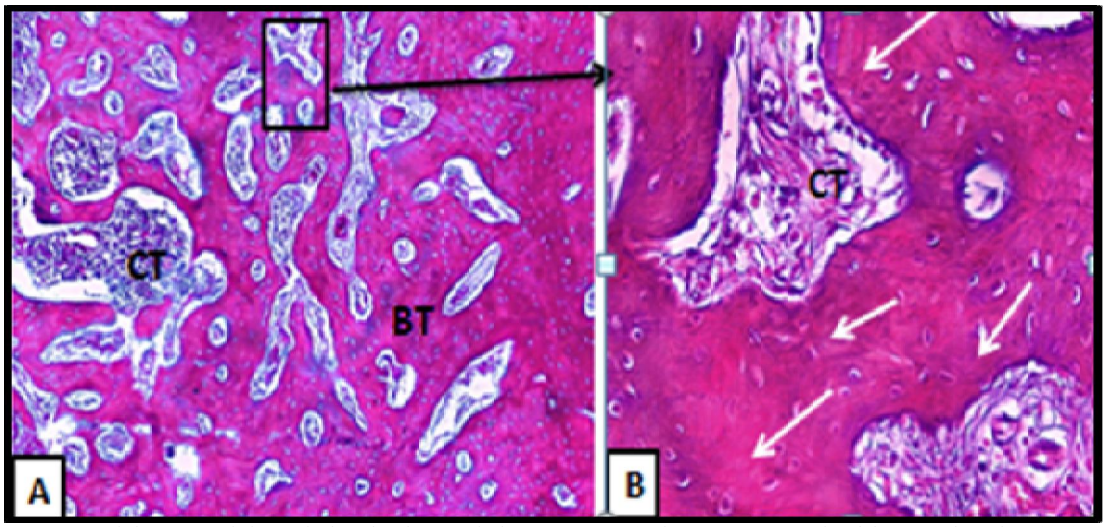

Fig. (6): Masson trichrome staining of alveolar bone of sham group, A) there were organized bone trabeculae (BT) (H & E, OMX100). B) Higher magnification of (A), specifically collagen and reddish-colored matured bone (white arrows), Connective Tissue (CT) (H & E, OMX200).

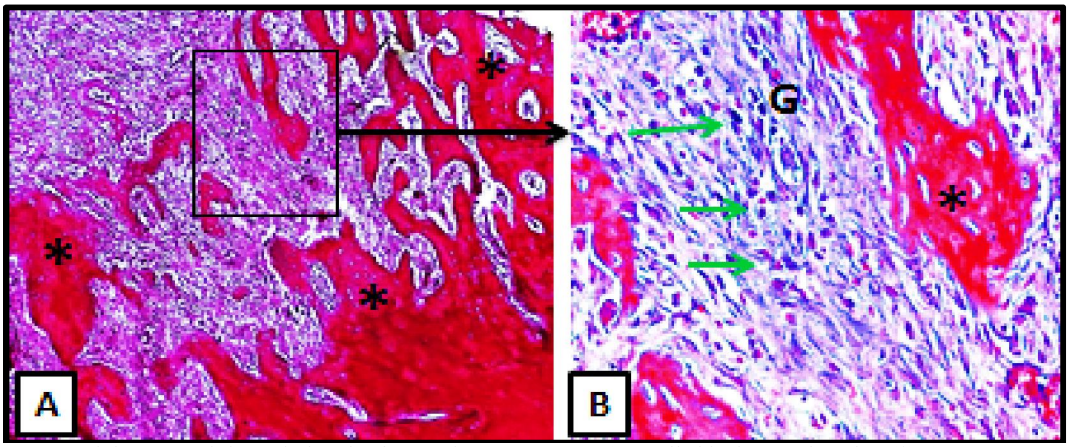

Fig. (7): Masson trichrome staining of alveolar bone of OVX group, A) small thin bonetrabeculae (*) (H & E, OMX100). B) Higher magnification of (A), small mature bone trabeculae formation was observed in small areas (*), granulation tissue (G) and infiltration of inflammatory cells (green arrows) (H & E, OMX400).

Table (1): Statistical quantitative analyses of newly formed bone in groups I & II.

	Group I (Control group)		Group II (OVX group)	
	the bone trabeculae	osteoclasts	the bone trabeculae	osteoclasts
Mean	397.6	3.4	106.2	17
S. D	10.99	2.30	11.99	6.16
Max	411	7	120	25
Min	385	1	89	11

Table (2): t-test of the means of the bone trabeculae & osteoclasts in control and OVX groups.

	Group I (Control group)	Group II (OVX group)	P-value
Bone trabeculae	389±10.99	106±11.99	0.000***
osteoclasts	3±2.30	17±6.16	0.001***

*** Very highly significant

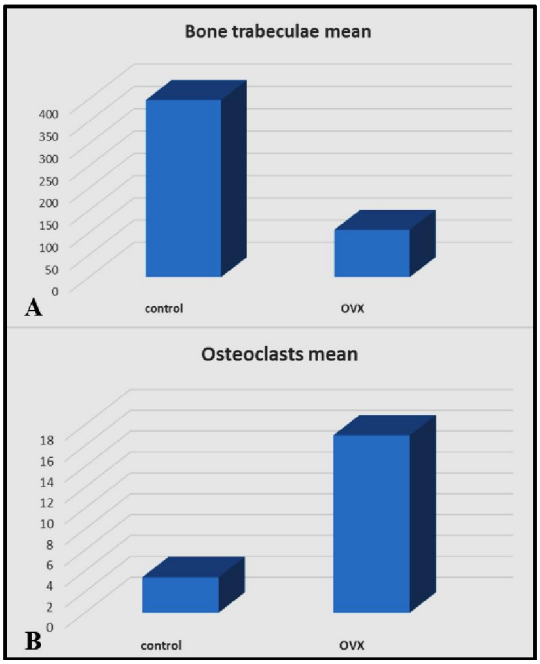

Figure (8): Column chart representing mean of the quantitative measurement of bone trabeculae & osteoclasts in groups I & II.

4. Discussion

Postmenopausal osteoporosis is long-lasting progressive skeletal bone illness disturbing physiology and biology of bone, along with long bones and jawbones of the body. The effect of osteoporosis on jawbones results in changes of alveolar bone which may negotiation the therapy and/or diagnosis of periodontal disorders [16,17].

The animal model for estimation of oesteoporosis represented in ovarectomized female rats is taken as standard golden model for evaluating drugs applied in the therapy or prevention osteoporosis; it has been well certified and resemble to several similar studies in adult humans suffering from bone loss caused by estrogen deficiency (or postmenopausal) [18]. Such similarities include: greater bone turnover; a preliminary period of rapid losses in bone accompanied by slowing of bone loss; and higher cancellous loss than in cortical bone [19]. So this study depended on OVX in female rats as animal model for osteoporosis that can replicate the postmenopausal period in women which characterized by estrogen deficiency.

In this study, LM results using H & E stained sections indicated few smaller bone trabeculae in the OVX female rats matched with sham rats. This coincidence with the bone trabeculae statistical quantitative measurement in which the greatest mean value was recorded in control group with the least value obtained in OVX group. This result may be due to drop in levels of estrogen in the circulation which reflect adversely on the procedures of alveolar bone remodeling in mandibles of ovarectomized female rats. This explanation coincidence with Payne et al [20] which indicated that a loss of alveolar bone density in postmenopausal women was associated with estrogen deficiency. Also confirmed by other studies in OVX animals, where estrogen is decreased to minimal concentration and cause induction of chondrogenesis and osteogenesis which are responsible for modification in the release of osteoinductive proteins like osteogenin and bone morphogenetic proteins, resulting in bone matrix formation changes [21-23].

Compared to the sham group, OVX group showed greater number of osteocytes were detected and their lacunae were larger and increased reversal lines. It was documented that estrogen levels have direct anabolic effects on bone cells [24,25]. OVX group showed granulation tissue which, in some areas, replaced by mature CT, characterized by angiogenesis and infiltration of inflammatory cells, these result agreed by Taubman and Kawai [26].

In our work, OVX revealed to the presence of deformity in the formation of trabeculae with fat-rich bone marrow-like tissue, these results are mimic to the finding of Yeung, et al. [27]. Enhanced bone marrow adipose tissue (BMAT) formation is also described in animal models of aging, OVX [28,29]. In post-menopausal osteoporosis, bone loss caused by bone transformation changes with either reduced number and/or mineralizing utility of osteoblasts, or with enlarged osteoclastic bone resorption that overcome bone development. BMAT is assumed to donate to this disturbed bone alteration via the adipogenesis procedures *per se* or its paracrine activity [30].

In the current study, osteoclasts in OVX group were recognized along the alveolar bone proper margin and around the small bone trabeculae compared with sham group. This result confirmed by the quantitative measurement osteoclast cells counting were highly significant elevated in OVX group than control one. Several researchers tried to clarify the precise cellular mechanisms for explaining osteoclastogenesis through the OPG / RANK / RANKL system [31]. In fact, the RANKL facilitates early postmenopausal women bone resorption [32,33]. Kawamoto et al. [34], suggested osteoclastogenesis was occurred in the periodontium of ovarectomized female rat, proposing that alveolar bone breakdown detected in the ovarectomized rats as a result of increased osteoclasticaction.

It is known that T cells are any of the main sources of the RANKL, which regulates osteoclast formation [35]. Garcia-Perez et al., [36] showed that RANKL+ / CD3+ T cells of bone marrow are increased. This means that the T cell capacity to control osteoclast differentiation differentiates osteoclast precursors into osteoclasts. T-cell-mediated adaptive immune response may be hypothesized to predominate in OVX classes.

The antiresorptive effects is the mode of estrogen action in resorption of bone, as a minimum partially through augmenting the level of osteoprotegerin (OPG) expression against that of RANKL. The activity of osteoclasts which subsequently increase resorption can be induced within the imbalance between RANK/RANKL/OPG system [10].

In the OVX group, loss of continuity and resorption areas of cementum were noticed opposite to the normal structural integrity of cementum in control group. This effect on cementum may be attributed to the similarity of differentiation between osteoclasts and odontoclasts, and because they are similar in histological origin. Recently, investigators found that root resorption was intricate in both osteoclastogenesis and odontoclas to genesis through the OPG/RANK/RANKL arrangement [37]. Some areas can undergo necrosis even with a viable periodontal ligament, resulting in the loss of cells such as pre-cement oblasts and cementum [38].

Masson trichrome stain of OVX group compared with sham animals exhibited thin mature bonetrabeculae. Formation of bone tissue is observed in small areas. This finding was described and reinforced by another research [39]. Other study founded that OVX induced osteopenia and increased the indices of bone resorption and formation at day 14 after ovarectomy in the rat tibia [40].

Zhou et al [41] stated the deficiency of estrogen down regulates protein expression thus causing cytokines including TNF-a and IL-6 to be released that cause bone tissue damaging effect. Following ovarectomy in rats or in women called menopause, deficiency of estrogen deteriorates the balance activity between osteoblast and osteoclast like that at bone resorption outweighs bone formation resulting in high turnover bone loss [42]. Since this condition has been simulated in the current study, the deficiency of estrogen may elucidate the variations among ovarectomized (OVX) and sham group in inflammation and turnover procedures.

Overall, the results of the current study indicate that deficiency of estrogen can affect the microstructural of the alveolar bone and tooth cementum which my increase the risk of jaw bone

fracture especially in surgical procedure, also may cause premature tooth loss.

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