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EFFECT OF FISH OIL SUPPLEMENTATION ON ALVEOLAR BONE STRUCTURE IN RATS WITH GLUCOCORTICOID INDUCED OSTEOPOROSIS (HISTOLOGICAL, IMMUNOHISTOCHEMICAL & ULTRASTRUCTURAL STUDY)

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ABSTRACT

Glucocorticoids are widely used in treatment of many medical disorders. However, many side effects are associated with the chronic use of glucocorticoids administration especially osteoporosis. Fish oil contains omega 3 polyunsaturated fatty acids which can inhibit the production of inflammatory mediators such as IL-6 and TNF-α which are important for osteoclastogenesis. So the aim of the present work was to test the efficiency of fish oil administration on alveolar bone osteoporosis induced by glucocorticoid administration. Materials & methods: 8 Wistar albino rats were divided into 3 groups. Group I: control group. Group II (osteoporotic group): rats were given 7 mg/kg of dexamethasone, once a week intramuscularly for 5 weeks. Group III (fish oil treated group): rats were given the same dose of dexamethasone like group II and at the same time were given daily an oral dose of fish oil 1gm/kg/day. All rats were sacrificed after 5 weeks from the start of the experiment and molar segments of the jaw were dissected out. They were examined histologically, immunohistochemically, histomorphometrically and ultrastructurally. Results: Histologically in osteoporotic group II the bone showed marked resorption with the presence of many osteoclasts which showed significant increase in number and intense TRAP immunostaining. By scanning electron microscope the buccal cortical plate showed obvious erosion. On the other hand, fish oil treated group showed marked improvement where the bone appeared denser with significant decrease in osteoclast number which exhibited mild TRAP staining. Histomorphometricly, the bone surface area showed marked increase. Ultrastructurally, the bone surface appeared relatively smooth. Conclusions: fish oil supplementation could to a large extent overcome the detrimental effect of glucocorticoid administration on the structure of the alveolar bone.

KEY WORDS: fish oil, osteoporosis, osteoclasts, alveolar bone

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INTRODUCTION

Glucocorticoids (GCs) are the drug of choice in the management of anti-inflammatory and immunosuppressive therapies. They are used in the treatment of a variety of disorders, such as autoimmune, pulmonary, and gastrointestinal diseases, in addition to certain chronic inflammations including rheumatoid arthritis, multiple sclerosis, psoriasis and eczema. Also, they are widely used in patients following organ transplantation and malignancies.[1, 2] However, chronic glucocorticoid therapy is accompanied with complications and toxicity. The skeletal system is the most prevalent system of the body having the potential to be severely affected by glucocorticoids; the most serious side effect is glucocorticoid -induced osteoporosis (GIO) as osteoporotic fractures affect 30-50% of patients.[3]

Different mechanisms underlie the catabolic effect of glucocorticoids on bone. First, they have a negative effect on osteoblastogenesis by impairing the replication and differentiation of osteoblasts, and by shifting the differentiation of undifferentiated mesenchymal cells away from the osteoblast pathway to adipocytes. Glucocorticoids also decrease the function of active osteoblasts through the inhibition of insulin-like growth factor I expression.[4] Indeed, osteocytes show decreased cell activity and marked apoptosis with accompanied inability to detect and repair bone micro damage.[5, 6]

Fish oil contains omega 3 polyunsaturated fatty acids (ω-3 PUFA) which are important for the general health. They are important components of the phospholipid bilayer in membranes allowing for intercellular communication and highly differentiated membrane functions. Indeed, they are the primary precursors of bioactive lipid mediators, which have autocrine and paracrine actions throughout the body.[7] These fatty acids include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have several potential health benefits in the prevention and treatment of cardiovascular disorders, some forms of mental illness, and inflammatory diseases.[8,9] Alpha linolenic acid (ALA) is a type of omega 3 present in many types of food including vegetables, seeds, nuts & soybean.[10] EPA and DHA can be obtained from fatty fishes or our body can convert ALA to EPA and DHA. However, this conversion is very small, so sufficient amounts of EPA and DHA should be ingested in the diet. [11-13]

ω-3 (PUFA) is a precursor for several potent regulatory lipid mediators known as eicosanoids including prostaglandins and leukotrienes which are involved in bone metabolism. Thereby, ω-3 PUFA can inhibit the production of inflammatory cytokines such as IL-1, IL-6, and TNF-α, which are important stimulatory factors for osteoclastic bone resorption. [14]

The immune-modulating and anti-inflammatory effects of ω-3 (PUFA) was first observed in cardiovascular disease, however the potent action of the inflammatory mediators traced in other conditions including metabolic bone diseases such as osteoporosis caused investigators to extend their studies of ω-3 (PUFA) to include skeletal outcomes.[7, 14]

A previous study by Galal et al (2014)[15] investigated the effect of omega 3 supplementation on chronic periodontitis in postmenopausal women with osteoporosis. They found that omega 3 can decrease pocket depth, increase attachment level and improve periodontal condition.

Most of the previous studies have been accomplished on the effect of fish oil supplementation on ovariectomy induced osteoporosis of female rats and mice.[16-18] Since, Glucocorticoids are frequently used in treatment of many medical conditions, so the aim of the present study was to investigate the efficacy of fish oil as a non-pharmacological way on osteoporosis which was induced by glucocorticoid administration.

MATERIALS AND METHODS

Experimental procedure

The study protocol was approved by the Ethical Committee of Faculty of Dentistry, Alexandria University. To calculate the accurate sample size, the power analysis and sample size calculation (PASS version 17) software was used. To achieve 80% power to detect a significant difference of 15% among groups with a 95% confidence interval (α=0.05), a sample size of 8 animals per group was chosen. The main outcome variable considered in this study was the change in total bone surface area.

Twenty four male Wistar albino rats 4 months old and weighing about 220-270 grams were used in this study. They were maintained in the Institute of Medical Research, Alexandria University. The animals were housed three rats per one metal cage under temperature 24± 2°C and light-dark periods of 12 hours. They were fed a standard diet with water ad libitum. Animals were handled following the rules provided by the guiding principles for the care and use of laboratory animals.[19] Rats were randomly allocated into three groups, 8 rats each.

Group I (**control group**): rats received daily 1 ml of saline orally.

Group II (**osteoporotic group**): rats were given 7 mg/kg of dexamethasone*, once a week intramuscularly for 5 weeks[20] , and received daily 1 ml of saline orally.

Group III (**fish oil treated group**): rats were given the same dose of dexamethasone like **group II** and at the same time were given daily an oral dose of fish oil** 1gm/kg/day by oral gavage syringe.[21]

Five weeks after the beginning of the study, all the animals were euthanized with a lethal dose (150 mg/kg body weight) of sodium thiopental. The mandibles were dissected out.

The molar region of the right half of the mandibles were processed for light microscopic examination, immunohistochemical and histomorphometrical analysis, while the molar region of the left half of the mandibles were processed for scanning electron microscope examination.

Light microscopic examination

The right halves of the mandibles were fixed in 10% neutral buffered formalin, rinsed in distilled water, decalcified in 8% formic acid, dehydrated in ascending grades of alcohol, cleared in xylene and then infiltrated and embedded in paraffin wax. From each specimen, 15 mesiodistal serial sections of 5 μm thickness were cut. The sections were stained with haematoxylin and eosin stain.[22]

Histomorphometrical analysis

Morphometrical analysis of the mean bone surface area in different groups was calculated using Image J 1.46r program. Three mesiodistal sections at different standardized depths were obtained from each specimen. From each section an image was taken at the same magnification power. In each image, three rectangles with standardized dimensions was drawn in 3 standardized regions which are: the upper left border, lower left border and the centre of the right border. The total surface area of each rectangle was recorded using image J by choosing measure from analyse tab. The surface area occupied by bone only was calculated by subtracting the area occupied by bone marrow from the total surface area of the rectangle (Fig.1). The results were expressed as percentage values (the proportion of area occupied only by bone tissue in relation to the total area of the standardized

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** Soft gelatin capsules, 1000 mg fish oil, the Arab Company for Gelatin & Pharmaceutical Products, Montana Pharmaceutical

rectangle). The procedure was repeated in each rectangle and the mean of the three rectangles was calculated. The same procedure was repeated in each of the three sections of the same specimen and the mean was obtained. The same steps were repeated in each of the 8 specimens in the different experimental groups. The results were expressed in terms of mean and standard deviation.

Immunohistochemical analysis

Sections were submitted to tartrate resistance acid phosphatase (TRAP) immunohistochemical staining. Sections were deparafinized in xylene and rehydrated in a descending grades series of alcohol to distilled water. They were immersed in 0.01 M citrate-buffer (pH =6) and then heated for 30 minutes in a steamer. Peroxide hydrogen (2%) was added for 10 minutes to block endogenous peroxidase and then washed with phosphate buffer solution (PBS). Afterwards, anti-TRAP primary polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, EUA) were incubated at 4^0 C overnight and then washed for 30 min, three times. The sections were incubated with biotinylated secondary antibody for 30 minutes, washed in PBS and then incubated for 30 minutes with streptavidin-peroxidase conjugate. Then, the reaction was stained with 3,30 -diaminobenzidine tetra hydrochloride (Sigma Aldrich, St Louis, USA) and counterstained with Mayer's hematoxylin. In negative control sections, primary antibody was omitted. [23]

Osteoclasts count

Counting of osteoclast number was done in TRAP stained sections. From each specimen, 3 mesiodistal sections were taken at different standardized depths. From each section, 3 photographs were taken (at the apical region of the alveolar bone surrounding the root apex) at the same magnification power. In each photograph, TRAP positive multinucleated cells adjacent to bone surface were counted as osteoclasts.[24] The same procedure was repeated in each photograph at the different standardized depths and the mean was obtained for each specimen. The same procedure was repeated for each of the eight specimens in each group and the mean was obtained.

Scanning electron microscopy

The left molar segments were separated from the rest of the mandibles. The specimens were then placed in 5% sodium hypochlorite solution for 2 hours for removal of all soft tissue. Then the specimens were fixed in 2.5% glutaraldehyde in phosphate buffer, washed under running water, dehydrated, and air-dried. Afterwards, the specimens

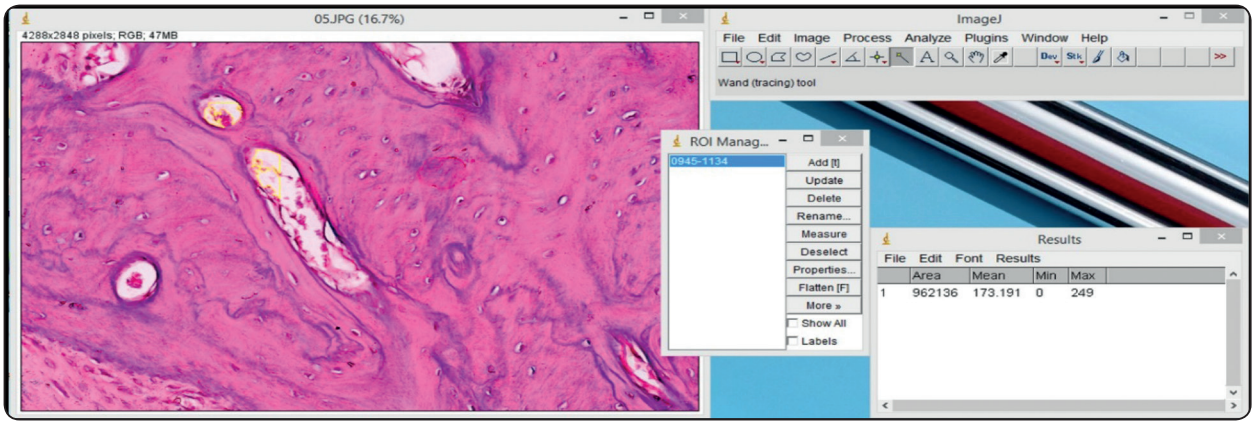

Fig. (1) The mean bone surface area is measured using Image J 1.46r software. The bone marrow spaces are traced in yellow using the wand tracing tool.

were coated with a thin layer of gold using a sputter coater to be examined on JOEL 5300 JSM scanning electron microscope. [25] The buccal cortical plate were examined in all specimens.

RESULTS

Light microscopic results

Histological examination of specimens obtained from control group revealed that bone consisted of numerous osteons with centrally located Haversian canals. Multiple resting lines were seen separating between the bone lamellae. Osteocytes exhibited regular distribution with large nuclei filling most of the osteocyte lacunae (Fig.2). On the other hand, in the osteoporotic group, pronounced bone resorption was evident. The bone trabeculae appeared thin surrounding wide bone marrow spaces. There was marked alteration in osteocyte distribution appearance. The osteocyte lacunae appeared irregularly arranged and many of them appeared with widened lacunae and pyknotic nuclei. Numerous osteoclasts were seen lining the bone surface facing the periodontal ligament and occupying Howship's lacunae. Reversal lines were also seen between the old and new bone (Fig.3). In fish oil treated group, marked improvement in bone structure occurred. The bone appeared dense with multiple reversal lines. Most of the osteocyte lacunae appeared with obvious nuclei, while some of them showed pyknotic nuclei. (Fig.4).

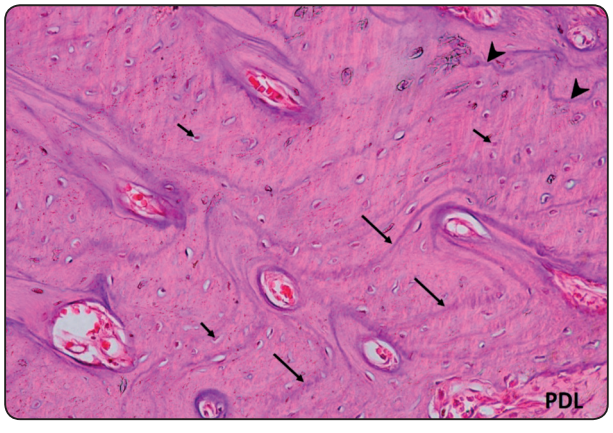

Fig. (2) Light micrographs (LM) of the alveolar bone in Control group I (A): The alveolar bone consists of dense bone with numerous resting lines (long arrows). Regularly distributed osteocytes (short arrows) and reversal line also can be seen (arrow head). PDL: periodontal ligament. (H&E 200x)

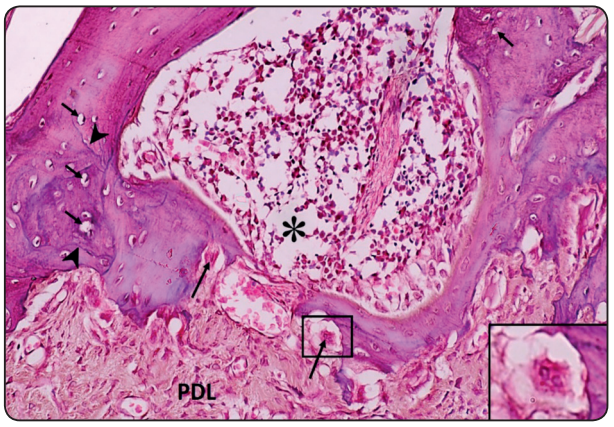

Fig. (3) LM of the alveolar bone in Osteoporotic group II (B): The bone shows marked bone resorption with widened bone marrow spaces (asterisk). The osteocyte lacunae are widened with pyknotic nuclei (short arrows). Osteoclasts in Howship's lacunae (long arrows) and reversal lines (arrow heads) can be seen. PDL: periodontal ligament. The inset shows higher magnification of an osteoclast. (H&E 200x, inset 400x)

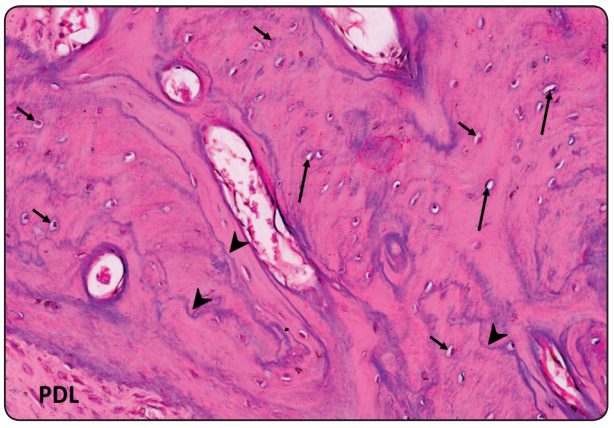

Fig. (4) LM of the alveolar bone in Fish oil treated group III (C): The alveolar bone appeared dense. The osteocyte lacunae are regularly distributed with obvious nuclei (short arrows), while others appeared with pyknotic nuclei (long arrows). Numerous reversal lines can also be seen (arrow heads). PDL: periodontal ligament. (H&E 200x)

Histomorphometrical analysis

The mean of percentage of bone surface area in different groups are summarized in table (1) by mean ± standard deviation.

There was a statistically significant decrease in mean percentage of bone surface area in osteoporotic group II in comparison to the control group I (p_1< 0.001) where values were 72.63 ± 3.85 & 88.0 ± 4.11 respectively. In the fish oil treated group III, there was significant increase in mean bone surface area in relation to the osteoporotic group II (p_3= 0.001) where values were 80.0 ± 4.04 & 72.63 ± 3.85 respectively. However, the difference between the fish oil treated group III and the control group I was statistically significant (p2 = 0.001) where the values were 80.0 ± 4.04 & 88.0 ± 4.11 respectively.

Immuno-histochemical analysis

Histological examination of TRAP immunohistochemically stained sections revealed very weak or almost absent staining of osteoclasts in control group I (Fig.5). A strong positive osteoclast staining was observed in osteoporotic group II (Fig.6), while in fish oil treated group, the osteoclast showed mild staining (Fig.7).

TABLE (1): Comparison between the studied groups according to bone surface area (%)

Bone surface area (%)	Control group I (n = 8)	Osteoporotic group II (n = 8)	Fish oil Treated group III (n = 8)	F	p
Min. – Max.	80.0 – 93.0	66.0 – 78.0	75.0 – 87.0	29.576*	<0.001*
Mean ± SD.	88.0 ± 4.11	72.63 ± 3.85	80.0 ± 4.04		
Median	89.50	73.50	79.0		
Sig. bet. Groups	p_1<0.001*, p_2=0.001*, p_3=0.001*				

***F,p: F and p values for* ANOVA test*, Significance between groups was done using* Post Hoc Test (LSD)**

p_1: p value for comparing between Control and Osteoporotic groups

p_2: p value for comparing between Control and Treated groups

p_3: p value for comparing between Osteoporotic and Treated groups

****: Statistically significant at p ≤ 0.05***

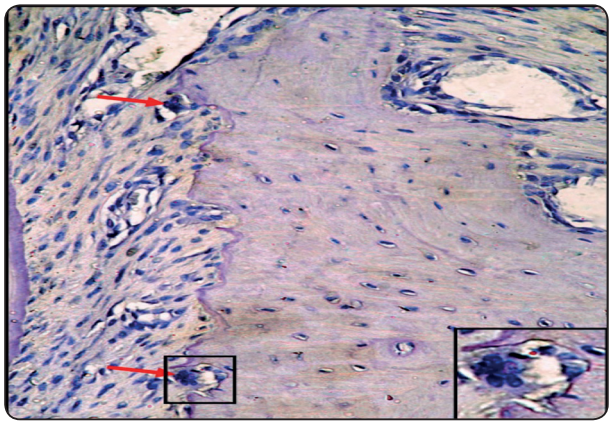

Fig. (5) LM of TRAP immunostaining of the alveolar bone of Control group I shows very weak staining in osteoclast cells (arrows). The inset shows higher magnification of an osteoclast with almost no staining. (200x, inset 400x)

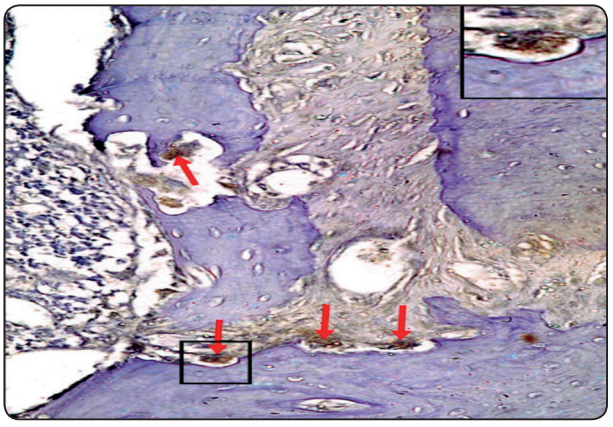

Fig. (6) LM of TRAP immunostaining of the alveolar bone of Osteoporotic group II shows numerous osteoclasts with strong TRAP immunostaining (arrows). The inset shows higher magnification of an osteoclast with deep staining. (200x, inset 400x)

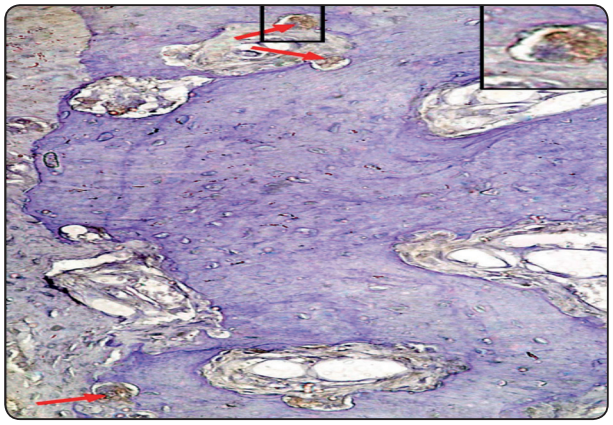

Fig. (7) LM of TRAP immunostaining of the alveolar bone of Fish oil treated group III showing mild TRAP positive staining in osteoclasts (arrows). The inset shows higher magnification of an osteoclast. (200x, inset 400x)

3.4 Osteoclast count

The mean osteoclast count in different groups are summarized in table (2) by mean ± standard deviation.

There was a statistically significant increase in mean osteoclast count in osteoporotic group II in relation to the control group I (p_1<0.001), where the values were 7.13 ± 2.23 & 1.25 ± 1.04 respectively).

The mean osteoclast count showed a significant decrease in fish oil treated group III in comparison to the osteoporotic group II (p3=0.030), where the values were 3.25 ± 1.83 & 7.13 ± 2.23 respectively. Moreover, the difference between fish oil treated group III & control group I were not significant (p2=0.072), where the values were 3.25 ± 1.83 & 1.25 ± 1.04 respectively.

Scanning electron microscopic results

Scanning electron microscopic examination of the buccal cortical plate of the control group revealed smooth and regular bone surface (Fig.8A). In osteoporotic group II, there was marked alteration of the normal surface structure of the mandible. Pronounced destruction of the buccal cortical plate was seen with the presence of deep resorptive craters (Fig.8B). On the other hand, in the fish oil treated group there was marked improvement in bone surface structure where the bone surface appeared relatively smooth and homogenous. However, there were shallow concavities can be still seen scattered on the bone surface (Fig.8C).

TABLE (2): Comparison between the studied groups regarding osteoclast count

Osteoclast count	Control group I (n = 8)	Osteoporotic group II (n = 8)	Fish oil Treated group III (n = 8)	H	p
Min. – Max.	0.0 – 3.0	3.0 – 10.0	1.0 – 7.0	11.091*	0.001*
Mean ± SD.	1.25 ± 1.04	7.13 ± 2.23	3.25 ± 1.83		
Median	1.0	7.50	3.0		
Sig. bet. groups.	p_1<0.001*, p_2=0.072, p_3=0.030*				

***H, p:** H and p values for* **Kruskal Wallis test,** *Sig. bet. grps was done using* **Post Hoc Test (Dunn's multiple comparisons test)**

p_1: p value for comparing between Control and Osteoporotic

p_2: p value for comparing between Control and Treated

p_3: p value for comparing between Osteoporotic and Treated

****: Statistically significant at p ≤ 0.05***

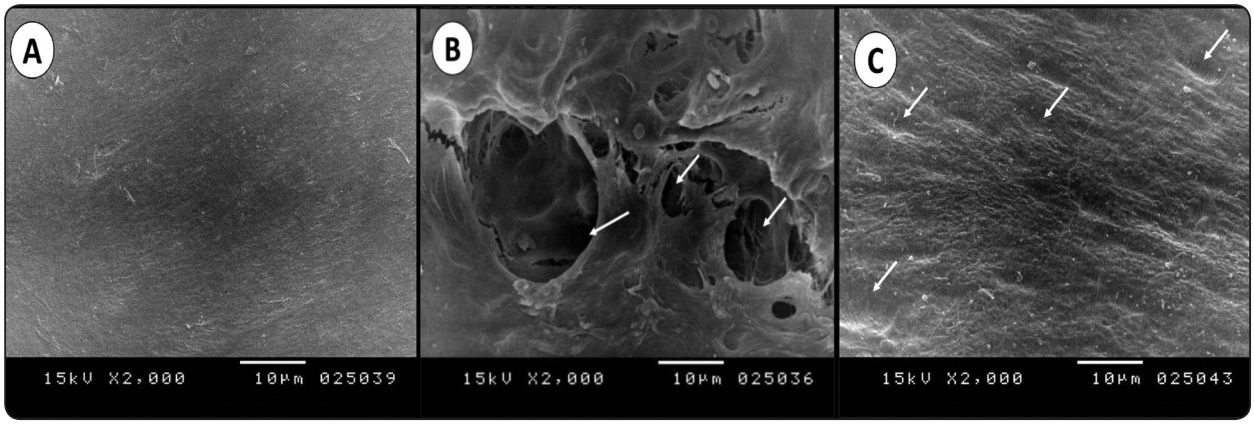

Fig. (8) Scanning electron micrograph (SEM) of the buccal cortical plate of the mandible in the different experimental groups. Control group I (A): The buccal cortical plate is smooth. Osteoporotic group II (B): Marked erosion of the buccal cortical plate with deep resorptive bays (arrows). Fish oil treated group III: The bone surface is relatively smooth with few shallow concavities (arrows) can be seen. (2000x)

DISCUSSION

Marine sources provide most of the ω-3 (PUFA) found in our diet, performing important physiological functions as they are incorporate into our body. For many years, they have been studied for treatment of cardiovascular disorders.[26, 27] The health benefits of these fatty acids may be attributed to their anti-inflammatory properties.[28, 29] Therefore, it has been speculated that they may play a role in other health conditions associated with inflammatory reaction such as osteoporosis.[30-33] So the aim of the current work was to investigate the effect of fish oil supplementation on osteoporosis of alveolar bone induced by glucocorticoid administration. This was accomplished using histological, immunohistochemical, histomorphometric and ultrastructural analysis.

Previous studies proved a correlation between systemic osteoporosis and mandibular bone density. Horner et al (1996) [34] found a correlation between bone mineral density of the mandibular body and other skeletal sites. In addition, Deguchi et al (2008) [35] confirmed a relation between thickness of mandibular cortex and serum markers of bone turnover. So mandibular bone can be a good indicator for general bone health.

In our work, osteoporosis was induced by administration of dexamethasone. Shefrin et al (2009)[36] stated that dexamethasone has a long half-life which is about 37-70 hours. Moreover it has a powerful effect about 6 times more than prednisolone.[37]

The light microscopic examination of the osteoporotic group II in the present study showed evident bone resorption with widening of the marrow spaces. Also, there was an increase in osteoclast number which were seen along the bone surface occupying Howship's lacunae. Moreover, the immunohistochemical analysis revealed strong TRAP immunostaining of the osteoclasts.

This is in accordance with Dovio et al & Swanson et al who found, through several studies, that glucocorticoids (GCs) have an immediate effect on osteoclasts. They observed a marked rising in bone-resorption markers in patients receiving intravenous GCs, also when bone explants were subjected to GCs, the level of osteoclasts markers showed significant increase 3 hours following the exposure. [38, 39]

These findings may be due to the fact that glucocorticoids have a direct stimulatory effect on the os-

teoclasts. They increase the expression of receptor activator of nuclear factor kappa ligand (RANKL) and colony stimulating factor 1 (CSF-1), and decrease osteoprotegerin (OPG) release from the surrounding osteoblasts. This switches the balance in favor of osteoclast maturation and activity. In addition, glucocorticoids have a direct anti-apoptotic effect on mature osteoclasts leading to increased osteoclast survival and activity. [40, 41]

The initial phase of bone resorption is accompanied by decrease in osteoblastogenesis through apoptosis of both osteoblasts and osteocytes and impairment in the production of type I collagen and bone stimulating factors such as insulin like growth factor 1 (IGF-1). These detrimental effects interfere with bone remodeling, with resultant predominance of resorption and loss of bone mass.[42]

In the present work, in the osteoporotic group II the osteocyte lacunae showed irregular distribution with enlarged lacunae and pyknotic nuclei. This is in agreement with Yao et al (2013) [43] who found that glucocorticoid (GC) administration resulted in an increase in the diameter of the lacunae, demineralization and reduction of elasticity of the matrix surrounding the lacunae. They also revealed that these altered osteocytes can produce proteins that downregulate osteoblast differentiation and matrix mineralization. In addition, Fowler et al (2017) [44] proved that GC administration to mice resulted in degeneration of canalicular connection between adjacent osteocyte lacunae, and collagen disorganization of the bone matrix.

In our study, histomorphometric results of the osteoporotic group II correlated with those of the light microscopic results. There was a statistically significant decrease in mean bone surface area in osteoporotic group II in comparison to the control group I ($p_1<0.001$). This is in accordance with Shomali et al (2009) [45] who found a significant decrease in trabecular thickness in the femoral epiphysis after glucocorticoid administration. Moreover, Bitto et al (2009) [46] demonstrated that glucocorticoid treated rats showed significant decreases in bone mineral density and bone mineral content of the femur neck.

Our scanning electron microscopic result of osteoporotic group II showed marked destruction of the cortical plates and disorganization of the bone surface. This is in agreement with Eberhardt et al (2001)[47] who reported that GC administration to rabbits resulted in marked bone resorption of trabecular bone of rabbits' femur which was examined by scanning electron microscope.

On the other hand, the light microscopic results of the fish oil group showed marked improvement in bone structure. The bone trabeculae appeared relatively thick containing multiple reversal lines. Histomorphometric analysis confirmed the histological results. There was a significant increase in mean bone surface area in the fish oil treated group III in relation to the osteoporotic group II ($p_3=0.001$). This is in coincidence with Matsushita et al (2008)[16] who concluded that fish oil can enhance the bone surface area of both cortical and cancellous bone in the femur of ovariectomized rats.

This may be due to the beneficial effect of ω-3 fatty acids on bone metabolism as it downregulates osteoclastogenesis through its anti-inflammatory properties. They inhibits the synthesis of tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2). [48, 49] Vanek and Connor also stated that dietary intake of ω-3 fatty acids reduces the production of Interleukin-1 (IL-1) and TNF-α in response to endotoxin stimuli. [50]

The effect of ω-3 fatty acids on osteoclastogenesis is coupled with osteoblastogenesis enhancement through suppression of parathyroid hormone activity and increase osteoblastic bone formation markers.[48] Watkins et al (2001) [31] stated that preosteoblastic cell line MC3T3-E1showed marked increase of the bone-formation markers such as alkaline phosphatase and osteocalcin after treatment

with ω-3 fatty acids. Another positive effect of ω-3 fatty acids on bone metabolism is that they can increase calcium absorption by increasing calcium ATPase activity [51, 52] and decrease urinary calcium excretion. [53] This is supported by previous studies which found a positive correlation between high fish oil intake and bone mineral density.[54 ,55]

In the present research, light microscopic examination of the fish oil treated group showed multiple reversal lines. These lines represent the line of demarcation between old and new bone. Previous studies revealed that these lines contain carbohydrates, low calcium content and osteopontin.[56, 57] Romano et al (1997)[58] proposed that these lines contain signaling molecules which can affect activity of osteoblasts. They revealed that in cases of improper bone healing, immature bone was formed instead of adult bone. Moreover, they found that in these cases alterations occur in the carbohydrate content of the reversal line.

Interestingly, the scanning electron microscopic results of the current research correlated with the histological studies. The fish oil treated group revealed marked improvement in bone surface structure as it appeared relatively smooth and homogenous, with few scattered shallow concavities. This confirm the positive effect of fish oil on bone metabolism.

The immunohistochemical results of our study of fish oil treated group revealed that osteoclasts showed mild TRAP staining. There was also a significant decrease in osteoclast count in relation to osteoporotic group III. This is in agreement with Ahmed et al (2013) [59] who investigated the effect of omega 3 on bone metabolism in salt loaded rats. They found that omega 3 treatment lead to decrease in osteoclast count and increase in bone thickness and plasma level of alkaline phosphatase. Moreover, Nakanishi et al (2013)[60] found that omega 3 supplementation decreased the number and activity of osteoclasts in ovariectomized rats. They suppress all key molecules necessary for osteoclastogenesis which include receptor activator of nuclear factor kappa (RANK), RANKL and monocyte colony stimulating factor (MCSF).

Histological examination of fish oil treated group in the ongoing study revealed relative normal distribution and size of osteocyte lacunae. This is in accordance with Morsy et al (2014)[48] who investigated the effect of fish oil on valproate (anticonvulsant drug) induced osteoporosis. They found that in fish oil treated group, there was an increase in number of osteocytes. Gao et al (2017)[61] stated that EPA can enhance bone cell survival and viability. They revealed that dexamethasone can induce apoptosis of bone marrow mesenchymal stem cells of rats, while EPA can increase the survival of these cells.

From the present study we can conclude that fish oil supplementation can play an important role in protection against secondary osteoporosis induced by glucocorticoid intake. The positive effect of fish oil on bone metabolism is mainly through inhibition of osteoclastogenesis and enhancement of bone formation.

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