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EVALUATION OF SYSTEMIC OMEGA-3 PUFAS EFFECT ON ORTHODONTIC TOOTH MOVEMENT IN A RABBIT MODEL: RCT

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Abstract

Objective: *is to evaluate the effect of systemic administration of Omega-3 fatty acids on orthodontic tooth movement (OTM) with histological analysis.* ***Materials and Methods:*** *OTM was induced in 20 adult albino New Zealand rabbits with NiTi coil spring for 21 days. Omega-3 or Saline was given every day via oral gavage during experimental period. Animals were sacrificed for histomorphometric analysis of alveolar bone remodeling after 21 days of OTM.* ***Results:*** *A significant difference in OTM amount was found in the 3rd week of OTM with means (1.445 ± 0.13), (1.72 ± 0.15) for experimental and control groups, respectively. Histomorphometric analysis showed a significant reduction in the area of active bone-resorptive lacunae and a significant decrease in osteoclastic activity related to omega-3 group after 3 weeks. A significant difference in osteoclast count is found, omega-3 group about (0.21 ± 0.09) while control group about (0.33 ± 0.11).* ***Conclusions****: A strong evidence of the osteoclastic inhibitory effect of systemic Omega-3 had been found which decreased percentage and amount of OTM.*

Keywords: *Omega-3 – Oral gavage – OTM*

Introduction

Orthodontic tooth movement is characterized by remodeling changes which involve dental and paradental tissues. At the compression side of the periodontal ligament (PDL), bone resorption is performed by osteoclasts and at the tension side, bone deposition is achieved by osteoblasts[1]. Maintaining tooth anchorage during orthodontic treatment has challenged orthodontists since the beginning of orthodontic treatment. Conventional methods for improving tooth anchorage aim at redirecting such forces to skeletal structures or distributing them over a larger number of teeth. Franzen et al [2] found that orthodontic relapse and OTM are associated with similar cellular adaptations, such as increased osteoclastic differentiation in compression areas. Given this background, one could argue that endogenous or pharmacologic bone modulation to inhibit osteoclast resorption and promote osteoblast neoformation may have clinically relevant effects on the regulation of OTM and relapse. Recently, retention strategies are aimed at increasing alveolar bone density after cessation of orthodontic tooth movement or control of alveolar bone remodeling around tooth roots by influencing osteoblasts and/or osteoclasts activity to prevent tooth relapse.

For nearly four decades, PUSFs family has been studied extensively in relation to prevention and treatment of cardiovascular disease [3]. The health-promoting effects of omega-3 fatty acids (FAs) may be partially due

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to their immune-modulating and anti-inflammatory actions [4]. Although this was first described in cardiovascular disease, the potential role that inflammatory mediators play in metabolic bone diseases such as osteoporosis, has caused investigators to extend studies of n-3 FAs to include skeletal outcomes[5]. Different mechanisms contribute to these effects, including conditioning cell membrane function and composition, eicosanoid production, and gene expression [6]. Omega-3, a polyunsaturated fatty acid, is composed of α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 fatty acids show anti-inflammatory effects via decreasing the level of proinflammatory cytokines and inflammatory mediators such as arachidonic acid–derived eicosanoids [(Prostaglandin E2–(PGE2)] [7, 8]. In a rat study model, it has been shown that omega-3 fatty acids inhibit osteoclast activity and bone resorption while they stimulate osteoblast activity and new bone formation [9]. Another review declared that n-3 and n-6 PUFA play a role in bone development and that n-3 PUFA may improve bone health by increasing calcium absorption in the gut, and increasing osteoblast differentiation and activity, reducing osteoclast activity and promoting deposition of mineral in developing bones [10].

There are few studies in the literature investigating the effect of omega-3 fatty acids on orthodontic tooth movement. Iwami-Morimoto et al[11] found that diet containing high omega-3 PUFA ratios decreased experimental tooth movement in rats than that rich in omega-6 FAs[12]. Kokkinos et al[13] reported that the concentration of PGE2 and arachidonic acid in alveolar bones of rats fed with fish oil was lower than rats fed with corn oil. In addition, Ogrenim et al[14] concluded that systemic administration of omega-3 fatty acids showed antioxidant and anti-inflammatory effects with deceleration of OTM. This paucity of information about omega-3 effect in orthodontics indicated the need for a more structured research approach, in both animal models and humans, to provide clinicians with more evidence-based results.

In this study, we used an experimental rabbit model to explore the effect of systemic administration of omega-3 on OTM. It was hypothesized that the osteo-inductive effects of omega-3 on the dental supporting tissues as well as the inhibition of osteoclastic activity might decrease OTM in rabbits. The research null hypothesis was that omega-3 supplement has no effect on OTM.

Material and Methods

Twenty adult male New Zealand albino rabbits (12 to 16 weeks old with body weight about 2.8 to 3.2 kg) were used for the experiment in the animal house of institute of graduate studies and research, Alexandria University, Egypt. Throughout the whole study period, animals were examined daily by the veterinarian staff for evaluation of the general health status of each animal, weight loss, appliance breakage, gingival or soft tissue inflammation. The animals were maintained at room temperature between 20 º and 25º C with constant humidity, fed with standard ground ration and water. All procedures involving animals were in strict accordance with

ARRIVE guidelines[15] for conducting animal studies and approved by the Ethics committee of the Faculty of Dentistry, kafrelSheikh University in Egypt, which includes the institutional experimentation committee, approved the research protocol.

Rabbits were divided into 2 groups, group I (10 animals) served as study (Appliance + Omega-3) and group II as control (Appliance + normal saline). All experimental procedures were performed under general anesthesia to maximize accessibility during operation. Ketamine was injected intramuscular at a dose 50mg/kg (Ketamine Alfasan 10%; Alfasan, Woerden, The Netherlands) and Xylazine (Xyla-ject Injectable Solution; ADWA, 10th of Ramadan City, Egypt), a muscle relaxant, was administered in the same manner at a dose of 5 mg/kg for appliance placement. Adequate depth of anesthesia was determined by visual inspection of tongue reflex when a dental mirror was inserted in the oral cavity.

Ligature wire (0.09 mm) was passed interdentally between first and second molars and wrapped around the first molar, similarly ligature wire also tied in figure of eight manner around incisors, and twisted with artery forceps till they fit into the grooves, then NiTi coil springs were tied to ligature wire between mandibular molar and incisors with 100 gram force using tensiometer (Morelli Orthodontic Tension Meter Force Gauge Intra/Extra Oral Elastics, Brazil) [16, 17]. A thin coat of flowable composite (Z350 XT flow, 3M ESPE, Calif, USA) was applied and light cured in order to avoid dislodgement of the appliance and lessen irritation of any wire projections. (Fig.1)

A piece of ligature wire was used to ligate the second molar to the third one to prevent any possible movement of the second molar mesially by the effect of the gingival interseptal fibers. (Fig.2A)

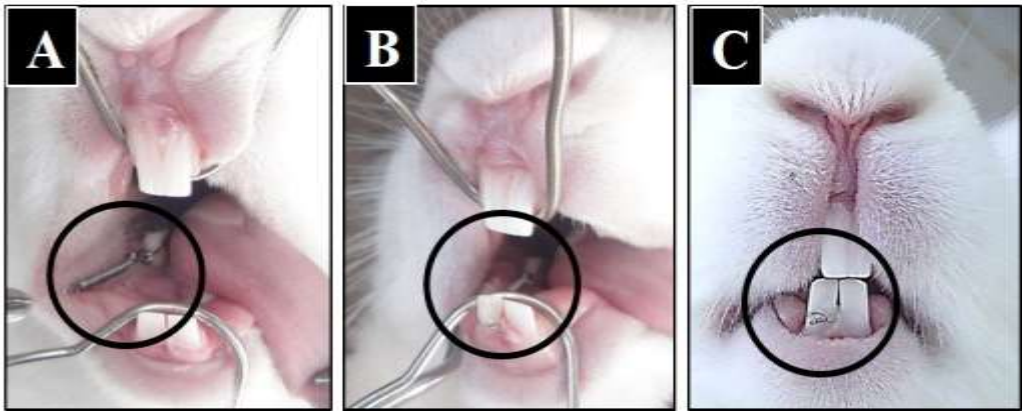

Figure.1. Intraoral photographs showing (a) ligation of coil spring to first molar tooth (b) ligation of coil spring to the incisor and (C) light cure flowable composite application

Figure.2A-B. (A) dissected rabbit mandible showing the appliance design and the diastema between molars after 21 days of active orthodontic tooth movement and (B) Impression making with light body Vinyl Polysiloxane with custom made special tray.

Rabbits were equally and randomly assigned to: (A) control group receiving saline or (B) experimental group receiving omega-3 by oral gavage daily (200 mg/kg)[18, 19] from day one of OTM.

Impressions of experimental teeth were performed on the 7th, 14th, and 21st days with the use of injection type silicone vinyl polysiloxane impression material (3M ESPE Express Vinyl Polysiloxane Impression Material—Fast Set; 3M ESPE Dental Products, Saint Paul, Minn) loaded into previously fabricated custom trays. The impressions were then poured with the use of an improved die stone (Elite Rock Dental Stone, Zhermack, Badia Polesine, Rovigo, Italy).

The intermolar distance (IMD) was measured manually with standard metric scale from the mesiooccusal margin of the second molar to the disto-occlusal margin of the first molar using a digital caliper with accuracy of 0.01 mm. Measurements were performed in a blinded fashion by a single investigator. The intraexaminer errors for tooth movement measurements were assessed by repeating the measurements 2 weeks apart by the same investigator.

After 21 days of OTM, animals were sacrificed and their mandibles were dissected, cut into halves, fixed, and decalcified. Parasagittal serial sections of 6 mm thickness were obtained, and 5 randomly selected sections per specimen were processed. The sections were stained with hematoxylin and eosin. Sections along the mesial aspect of the root of the mandibular first premolar in each group were evaluated under a light microscope (Zeiss Primo Star Light Microscope; Carl Zeiss, Oberkochen, Germany) equipped with a 5-megapixel digital camera, and images of representative areas were captured and described.

Statistical Analysis

The statistical analysis was accomplished using Statistical Package for Social Sciences SPSS software (IBM SPSS Statistics for Windows, version 23; IBM, Armonk, NY) for conducting statistical analysis. For comparisons between

different groups, Kolmogorov-Smirnov and Shapiro-Wilk tests were used to verify the normality of distribution. Once verified, paired-samples t-test was conducted to compare mean values between different experimental groups. Otherwise, Wilcoxon signed ranks test was conducted.

Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level, differences with P-values less than 0.05 was considered significant.

Results

Clinical Results:

After 3 weeks of force application, tooth movement of the first molars ranged from 1.3 mm to 1.9 mm, with a mean movement of 1.6 ± 0.3 mm. The amount and percentage of OTM through the three weeks are demonstrated in tables (1) and (2), respectively.

OTM (mm)	Experimental (n = 10)	Control (n = 10)	t	p
1st week				
Min. – Max.	0.5 – 0.75	0.5 – 0.8		
Mean ± SD.	0.63 ± 0.20	0.69 ± 0.10	2.260	0.12
Median	0.65	0.74		
2nd week				
Min. – Max.	0..9 – 1..32	0.9 – 1.2		
Mean ± SD.	1.07 ± 0.16	1.12 ± 0.15	2.26	0.65
Median	1.1	1.1		
3rd week				
Min. – Max.	1.2– 1.7	1.32 – 1.9		
Mean ± SD.	1.44 ± 0.13	1.72 ± 0.15	2.2	0.003*
Median	1.43	1.73		

Table (1): A comparison between the two studied groups according to amount of OTM (mm).*: Statistically significant at $p \leq 0.05$

IMD (%)	Experimental	Control	t	p
1st week of OTM				
Mean ± SD.	43.75 ± 1.1	40.1 ± 2.63	0.600	0.554
2nd week of OTM				
Mean ± SD.	30.55 ± 1.01	25.5 ± 3.61	2.270	0.022
3rd week of OTM				
Mean ± SD.	25.69 ± 1.50	34.88 ± 2.90	2.362*	0.017*

Table (2): A comparison between the two studied groups according to percentage of OTM per week. *: Statistically significant at $p \leq 0.05$

In the first week, the amount and percentage of OTM in both control and experimental groups were nearly the same, (0.69 ± 0.1 mm; 40.01 %), (0.63 ± 0.07 mm; 43.75 %).

were nearly the same (1.1 ± 0.01 mm; 25.5 %), (1.07 ± 0.15 mm; 30.55 %).

In the third week, the amount and percentage of OTM in experimental and control groups were (1.44 ± 0.13; 25.69 %) and (1.72 ± 0.15; 34.88 %), respectively.

Histological Results:

Presence of a large number of active osteoclasts in the mesial surface of the socket was observed in control group (Fig.3 & 4) which was in contrast with experimental group that showed decreased in the number multinucleated osteoclasts with reduction in the number and extent of bone resorption lacunae, table (3).

In the second week, the amount and percentage of OTM in both control and experimental groups

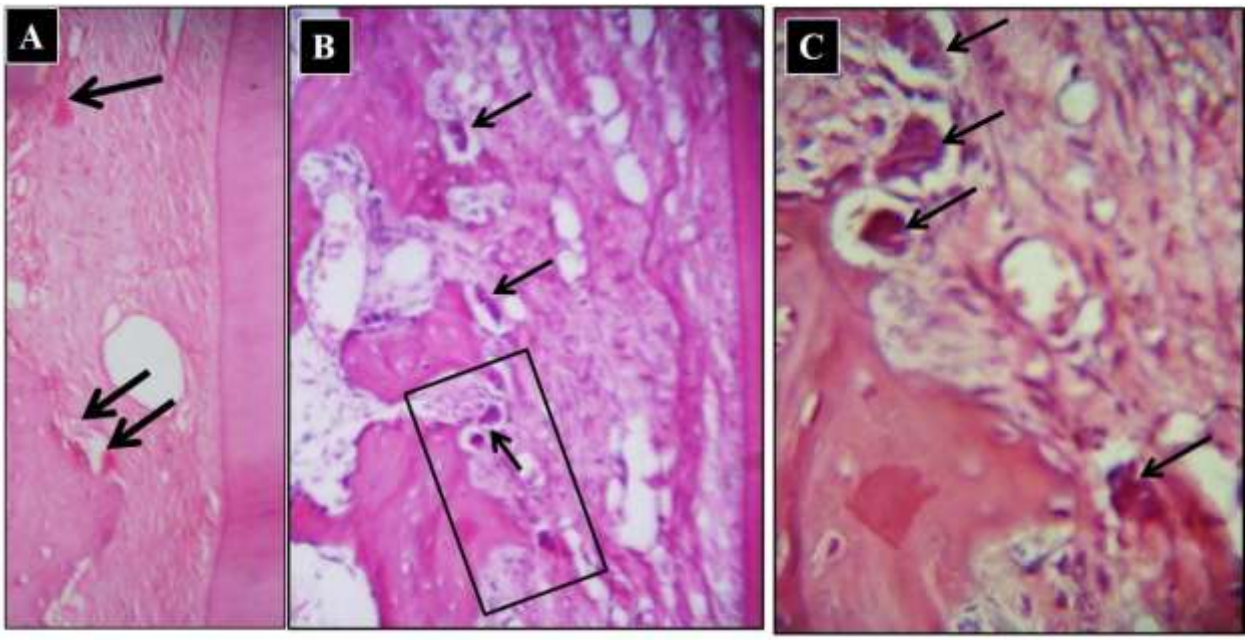

Figure.3A-C. Light microscopic images from mesial surface (compression) of first mandibular molar in control group showing: (A) the irregular surface of the alveolar bone with extensive bone bone-resorptive lacunae, [H&E stain, X100], and (B & C) highly active voluminous osteoclasts, [H&A, X400].

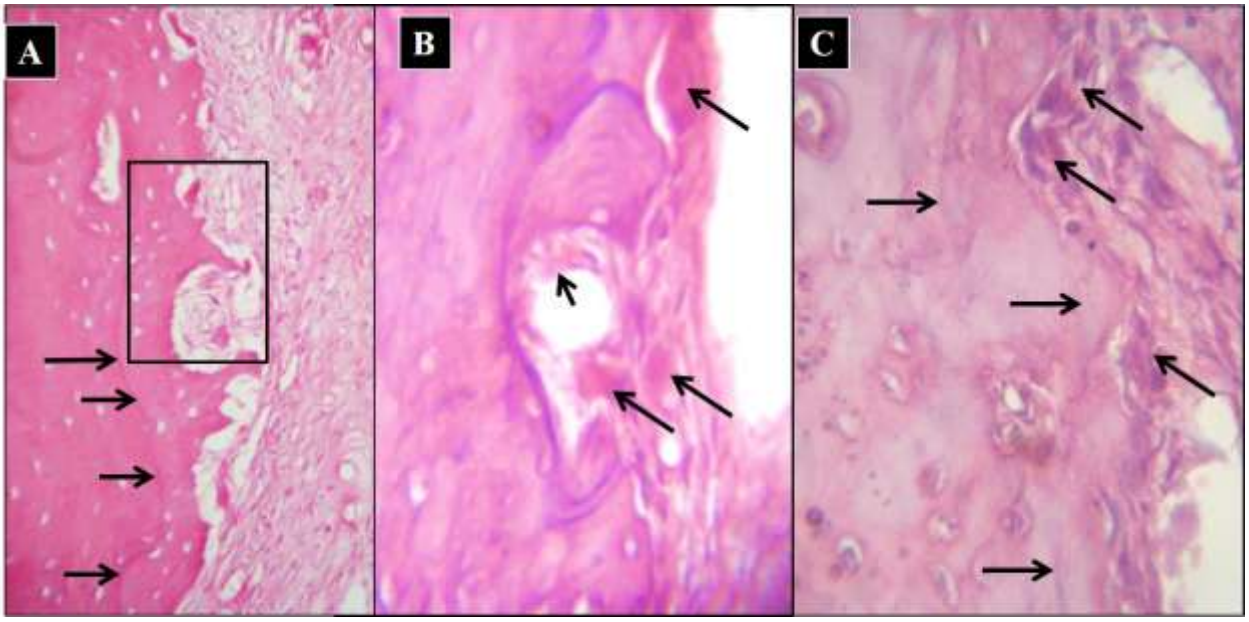

Figure.4A-C. Light microscopic images from mesial surface (compression) of first mandibular molar in experimental group showing: (A) the irregular surface of the alveolar bone with shallow bone-resorptive lacunae, [H&E stain, X100], and (B &C) small osteoclast, [H&A, X400].

Parameter	Control	Experimental	Test of sig	P
Osteoclast Count	4.33 ± 0.49	2.33 =± 0.98	2.14	0.0008*
Area of active bone-resorptive lacunae	0.33 ± 0.11	0.21 ± 0.09	2.1	0.002*

Table (3): A comparison between the osteoclast count in control and experimental groups after 21 days of OTM

Discussion

Omega-3 PUFAs are essential to normal growth and health. Recently, a strong relation was found between these acids, bone health and bone formation. The former was found to affect bone formation, bone resorption, serum calcium and inflammatory mediators, but the exact mechanism of action has not yet been determined[20].

The rabbit is one of the most widely used models for studying bone remodeling. In the current study, the rabbit model was chosen, in comparison with other species, such as primates and some rodents, as rabbits have faster skeletal change and bone turnover (significant intracortical, Haversian remodeling) [21].

Tissue reactions to orthodontic forces in adult humans start within 2 days after force application, whereas in rodents, tissue reactions start within 30 minutes of force application[22]. Kilic et al[23] showed that tooth movement in rabbits occurred in 3 phases: initial phase, arrest or lag phase, and acceleration or progressive movement phase.

The spring design and the active tooth-movement period of 21 days were chosen in this study in accordance with other studies with rabbits to allow proper time for systemic effect of omega-3[24, 25]. The orthodontic appliance used in the present study exerted an orthodontic force of 100 cN. This was in accordance with the force level previously used for inducing orthodontic movement of mandibular molars in a rabbit model [24]. The intermolar distance was measured with a digital caliper on stone casts because it was believed that it would be repeatable, easier and more accurate than doing it directly while the animal was under anesthesia.

Furthermore, the daily Omega-3 dosage (200 mg/kg) was selected based on previous animal studies recommending this amount as an optimal dose without adverse effect. Previous studies showed that higher doses of omega-3 (400 – 600 mg/kg) supplementation can lead to

bone resorption and imbalance in minerals of bone[18, 19].

In the first week of OTM, almost half of the total OTM percentage was found in both groups (experimental group 43.75 % and control group 40.01 %), followed by 30.55% and 25.5 %, respectively. The initial increase in amount of tooth movement during the 1st week, followed by a decrease in the amount of tooth movement during the 2nd week is in agreement with previous studies by Iwami-Morimoto et al [11] and Kokkinos et al [13].

A significant decrease of OTM was found in the experimental group rather than in the control group after three weeks of OTM. This delay of action was due to oral gavage administration which was in agreement with Azuma et al[26] and Al-Hashimi et al[19].

Histologically, the appearance of the osteoblasts in experimental group either on the mesial or distal surface of the socket was noticeably greater than their appearance in control group. On the other hand, osteoclasts were noticeable smaller, less frequently encountered and less active than those observed on the same surface of the control teeth. Iwami-Morimoto et al [11] showed that fish oil enriched diets reduced osteoclastic activity and the amount of alveolar bone resorption on the pressure side which was in agreement with our study and other studies in mice[27], rats[18] and humans[28].

In addition, all previous studies that had examined the effect of dietary lipids on alveolar bone remodeling or orthodontic tooth movement, are consistent with this study results about the inhibitory effect of omega-3 on alveolar bone resorption. Alam et al [29]found a relation between the type of dietary lipids and the fatty acid composition of bone lipids. Kokkinos et al [13] revealed that omega-3 enriched diet had inhibitory effect on OTM. Both studies concluded lipid diets induced changes in archidonic acid level in alveolar bone with accompanied changes in PG levels. Moreover, our study used oral gavage to deliver and assure the intake of proper amount of omega-3 supplement.

The current study revealed that osteoclast count and OTM are directly correlated, highlighting the importance of bone remodeling during OTM and relapse phases. This was in agreement with the study done by Dolci et al [30] who concluded that Statin-induced OPG overexpression reduced relapse after OTM, in a phenomenon correlated with decreased osteoclast counts. However, considering overall osteoclast number and OTM findings, we observed that the control group had high osteoclast count with high activity in compression site, whereas the omega-3 group exhibited the opposite profile. Once, the essential elements (osteoclastic activity and number and diminished cellular infiltrate) for bone remodeling are reduced, the OTM or even relapse is affected.

Conclusion

The results of the present study indicate that systemic administration of omega-3 PUFAs could reduce the amount of OTM due to its osteoclastic inhibitory effect.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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