Biochemical Assessment of Olive Oil Consumption During Orthodontic Retention Period

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ABSTRACT

This study attempted to evaluate biochemically the effects of chronic olive oil consumption on orthodontic relapse after orthodontic retention period. So as to, present a supplementary strategy based on dietary resources that may help to maintain orthodontic retention results and reduce orthodontic relapse tendency. . Materials and Methods: Thirty apparently healthy female albino rabbits, weighting more than 1000 gm each were used in this study. The animals were grouped randomly into 6 groups of 5 animals in each, 2 control groups and 4 olive oil groups. A modified fixed orthodontic appliances were fixed to rabbit lower central incisors. Each rabbit received orthodontic intervention for one week followed by six weeks retention period. At the end of the experiments, the rabbits were sacrificed and blood samples were collected for serum samples. Rabbit Matrix metalloproteinase-2, Rabbit Bone Specific Alkaline Phosphatase and Rabbit Tartrate Resistant Acid Phosphatase 5b ELISA kits were used for biochemical assessments. Data analyses were performed at level of P<0.05 for the statistically significant difference. Results: Biochemically, olive oil low concentration zero time group showed the highest Matrix metalloproteinase-2 value, while olive oil high concentration four weeks group revealed the lowest Matrix metalloproteinase-2 value. The highest Bone Specific Alkaline Phosphatase value was found in olive oil high concentration zero time group, whereas the lowest Bone Specific Alkaline Phosphatase value was for control four weeks group. The highest Tartrate Resistant Acid Phosphatase 5b value was found in control zero group, whereas the lowest Tartrate Resistant Acid Phosphatase 5b value was found in olive oil low concentration zero time group. Conclusions Supplementation with olive oil during orthodontic retention period, especially at 15.4 ml/kg b.w./day concentration reduced orthodontic relapse by biochemically increasing Bone Specific Alkaline Phosphatase, reducing Matrix metalloproteinase-2 and reducing Tartrate Resistant Acid Phosphatase 5b activities at end of four weeks after orthodontic retention period. Supplementation with olive oil at 7.7 ml/kg b.w./day concentration, biochemically increased Matrix metalloproteinase-2, increased Bone Specific Alkaline Phosphatase, and reduced Tartrate Resistant Acid Phosphatase 5b activities at end of orthodontic retention period.

Key words: Bone Specific Alkaline Phosphatase, Matrix metalloproteinase's, Olive oil, Orthodontic relapse, Orthodontic retention, Tartrate Resistant Acid Phosphatase 5b.

INTRODUCTION

After orthodontic treatment(OT), there are both a retention (R) phase and a post-R phase of therapy. The goal of orthodontic R is to increase the stability of the dentition after OT [(1)]. The post-R phase, begins when increased remodeling of the supporting tissues after OT is complete and lasts the rest of the patient's life. The goal of the post-R phase is to maintain the alignment of the dentition regardless of changing forces [(2)]. Unfortunately, in the post retention phase, orthodontic relapse may occur complicating the results after orthodontic retention period. Many factors are attributing to orthodontic relapse [(1,3)]. On the other hand, many attempts have been done to overcome the problem of orthodontic relapse [(3, 4)].

Olives and olive derived are an important part of Mediterranean diet. Olive oil (Ol), extracted solely from the fruit of olive trees (Olea europaea L [(5)]. In addition to its mono- unsaturated fatty acids, as oleic acid [(6)], Ol provides a variety of minor compounds or microcomponents that attribute to the quality of olive oil, with beneficial properties [(7)]. The active components include hydrocarbons (as squalene), sterols (asβ-sitosterol), polyphenols (tyrosol, hydroxytyrosol, oleuropein and many others), tocopherols, terpenoids, and traces of other constituents [(5)]. Olive oil is a source of at

least 36 structurally distinct phenolic compounds [8], whose total concentration ranges from 0.02 to 600 mg/kg [9], all acting as strong antioxidants, radical scavengers and non-steroidal anti-inflammatory -like drugs (NSAIDs)[10]. Furthermore, studies have been shown that olive oil has beneficial health effects [7,11]. Several studies revealed positive effects of Ol to bone health ([12,13,14].

Matrix metalloproteinases(MMPs), also called matrixins, are endopeptidases that are zinc-dependent and responsible for breaking the peptide bonds of amino acids. The MMPs belong to a larger family of proteases well-known as the metzincin superfamily[15]. Matrix metalloproteinases are illustrious from other endopeptidases by reliance on metal ions as cofactors, ability to degrade extracellular matrix (ECM), and specific evolutionary DNA sequence. To date, about 30 MMPs have been recognized [16],and 24 genes have been identified in humans that encode different groups of MMPs enzymes [17]. Matrix Metallopeptidase-2(MMP-2)also known as collagenase type IV-A, CLG4A collagenase Type IV, 72-KD, gelatinase, 72-KD, gelatinase A, gelatinase, neutrophil [17], is an enzyme that in humans is encoded by the MMP-2gene[18]. Matrix Metallopeptidase-2 more specifically hydrolyze denatured collagens (gelatin), native types IV (the major structural component of basement membranes), V and XI collagens, and elastin [19].

Aims of the Study: The main objective of this study was to find out an auxiliary aid based on natural dietary sources that is available in human diet to develop a supplementary strategy that may help to maintain retention results following OT and to reduce orthodontic relapse tendency.

The specific objectives of this study were to:

- Specify biochemically the influence of Ol on MMP-2 enzyme, Bone Specific Alkaline Phosphatase (BAP), and Tartrate Resistant Acid Phosphatase 5b (TRAP 5b) activity after orthodontic retention period.

Hypotheses

- Biochemically, there is a difference between MMP-2, BAP, and TRAP 5b of the control groups on one side and that of Ol groups on the other side.

EXPERIMENTAL

The Sample: Thirty apparently healthy female albino rabbits, weighting more than 1000 gm each were used in this study. The rabbits were kept in metallic cages in a well-ventilated room in the animal house of the College of Dentistry, University of Mosul. They were kept in the animal house for about two weeks for acclimatization before their use in the experiments.

Information concerning the rabbits' number, rabbit's group, weight, dose of anesthesia, dose of Ol, date of orthodontic appliance insertion, date of beginning of orthodontic R, date of orthodontic appliance removal, and date of sacrifice was recorded for each rabbit .

Diet adjustment for the total sample was made to exclude the possible effects of food's type on the rate of tooth movement. Water was available for the animals at any time.

The animals were grouped randomly, into 6 groups, of 5 animal each. Each animal received orthodontic intervention for one week followed by six weeks retention period. Animal grouping were as follow:

C0 group: the relapse was estimated at zero day after orthodontic retention period.
C4 group: the relapse was estimated at end of fourth week after orthodontic retention period.
Ol L0 group: received Ol, 7.7 ml/kg b.w./day during orthodontic retention period, the relapse was estimated at zero day after orthodontic retention period.
Ol L4: group received Ol, 7.7 ml/kg b.w./day during orthodontic retention period, the relapse was estimated at end of fourth week after orthodontic retention period.
Ol H0: group received Ol, 15.4 ml/kg b.w./day during orthodontic retention period, the relapse was estimated at zero day after orthodontic retention period.
Ol H4: group received Ol, 15.4 ml/kg b.w./day during orthodontic retention period, the relapse was estimated at end of fourth week after orthodontic retention period.
All the doses mentioned above were scheduled based on the study of Corona et al.[20] and according to guidelines set by American Food and Drug Administration [21] and dose conversion method of Shin et al.[22].

Olive oil Administration: Highly refined low acidity Ol (Sigma Aldrich, USA) was administered to the Ol groups via oral route through mixing to animal diet.

Orthodontic Intervention: A modified fixed orthodontic appliances were fixed to rabbit lower central incisors and exerted a reciprocal lateral force of 40±2 gms. Each rabbit received orthodontic intervention for one week followed by six weeks retention period.

Animal Sacrification, Collection and Processing of Blood Samples: At the end of the experiments, the rabbits were sacrificed and blood samples were collected from severed neck vessels at this time for serum samples collection to perform the biochemical assays.

Biochemical Assessments: Rabbit MMP-2 kit was a 1.5 hr solid-phase ELISA which applied the competitive enzyme immunoassay technique utilizing a highly specific monoclonal anti-MMP-2 antibody and MMP-2-HRP (Horse Raddish Peroxidase) conjugate for quantitative determination of rabbit MMP-2. To reveal the osteoblastic activity, the Rabbit BAP ELISA kit (MyBioSources Company, USA), which applies the competitive enzyme immunoassay technique utilizing a highly specific monoclonal anti-ALPL antibody and ALPL-HRP conjugate was used. To reveal osteoclastic activity, TRAP5b ELISA kit (MyBioSources Company, USA) ,which applies the sandwich enzyme immunoassay technique utilizing a highly specific monoclonal anti-TRAP5b antibody and TRAP5b-HRP conjugate was used.

Dataanalyses:were performed using the Statistical Package for Social Sciences Software (SPSS) for Windows (19.0)(SPSS Incorporated, Chicago, IL).

1. The data were checked for their normal distribution.
2. Descriptive statistics to show mean, standard deviation for each variable.
3. Non-parametric statistical approach were used. Median, interquartile range Mann-Whitney test was performed to analyze the differences among the study groups concerning clinical and biochemical parameters.
4. P-Value of less than 0.05 was considered a statistically significant difference.

RESULTS

Figure 1 shows the mean values for MMP-2, BAP and TRAP5b with various mean values among different groups. Group Ol H4 revealed the lowest MMP-2 value, while OL L0 group showed the highest MMP-2 value.
The highest BAP value was found in Ol H0 group, whereas the lowest BAP value was for C4 group. The highest TRAP5b value was found in C0 group, whereas the lowest TRAP5b value was found in Ol L0 group.

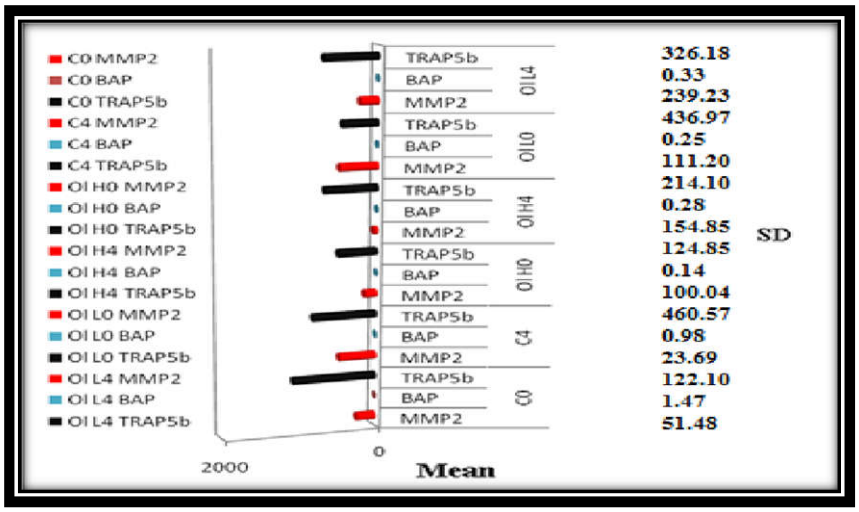

Fig. 1. Bar chart shows mean and standard deviation for the biochemical findings of the study groups. C 0: control zero time, C 4 : control 4 wks, Ol H0 : olive oil high dose zero time, Ol H4 : olive oil high dose 4 wks, Ol L0 : olive oil low dose zero time, Ol L4 : olive oil low dose 4 wks.SD is standard deviatioin; Variable unit for MMP2=pg/mL ; BAP= ng/mL ;TRAP5b= µU/ml.

Matrix Metalloproteinase-2: The result of this study showed no significant difference in majority of the values for group comparisons in statistical analysis. However, the OLH4 group exhibited a significantly lowest MMP-2 value than the remaining groups, and Ol L0 group showed significantly highest MMP-2 value (Table 1).

Table 1: Mean and standard deviation for the biochemical findings of the study groups.

Group	Variable	N	Mean	Standard deviation
C0	MMP2	5	241.81	326.18

	BAP	5	0.4012	0.33
	TRAP5b	5	1073.30	239.23
C4	MMP2	5	475.99	436.97
	BAP	5	0.34	0.25
	TRAP5b	5	819.99	111.20
Ol L0	MMP2	5	487.2256	460.57
	BAP	5	0.34	0.98
	TRAP5b	5	451.34	23.69
Ol L4	MMP2	5	228.98	122.10
	BAP	5	0.42	1.47
	TRAP5b	5	689.50	51.48
Ol H0	MMP2	5	150.3088	214.10
	BAP	5	1.2254	0.28
	TRAP5b	5	494.4062	154.85
Ol H4	MMP2	5	43.2126	124.85
	BAP	5	.6028	0.14
	TRAP5b	5	669.5714	100.04

C 0: control zero time, C 4: control 4 wks, Ol H0: olive oil high dose zero time, Ol H4: olive oil high dose 4 wks, Ol L0 : olive oil low dose zero time, Ol L4 : olive oil low dose 4 wks. Variable unit for MMP2=pg/mL ; BAP= ng/mL;TRAP5b= μU/ml.

Bone Specific Alkaline Phosphatase: For BAP, no significant difference was found between values for the various groups (Table 2).

Tartrate Resistant Acid Phosphatase 5b: Significant difference was found for most of the values in group comparison regarding TRAP5b (Table 3).

Table 2: Comparison of the Matrix Metalloproteinase-2 findings for the study groups.

Groups	Median	Interquartile Range	Mann Whitneytest	
			Cal.Z	P- value
C 0	293.78	337.12	1.15	0.251
C 4	373.30	457.77		
Ol L0	485.65	222.95	1.78	0.076
Ol L4	165.85	332.80		
Ol H0	119.14	96.04	2.61	0.009*
Ol H4	39.87	41.82		
C 0	293.78	337.12	2.19	0.028*
Ol L0	485.65	222.95		
C 0	293.78	337.12	0.52	0.602
Ol H0	119.14	96.04		
C 4	373.30	457.77	1.78	0.076
Ol L4	165.85	332.80		

C 4	373.30	457.77	2.61	0.009*
Ol H4	39.87	41.82		

C 0: control zero time, C 4: control 4 wks, Ol H0: olive oil high dose zero time, Ol H4: olive oil high dose 4 wks, Ol L0: olive oil low dose zero time, Ol L4 : olive oil low dose 4 wks.Cal.Z is Calculated Z; *Significant at ($p<0.05$); Variable unit for MMP2=pg/mL**.**

DISCUSSION

In the present study, MMP-2, BAP and TRAP5b are selected as biomarkers that are substances measured and evaluated objectively as indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [(23)]. In investigating biomarkers, the rate, amount, and activity of the released substances not only reflect the activity of individual cells but also indicate the metabolic activity in the involved tissues or organs [(24)].

In the current study, the most obvious results were that group Ol H4

Table 3: Comparison of the Bone Specific Alkaline Phosphatase findings for the study groups.

Groups	Median	Interquartile Range	Mann Whitneytest	
			Cal.Z	P- value
C 0	0.46	0.45	0.31	0.754
C 4	0.27	0.39		
Ol L0	0.15	0.58	0.73	0.465
Ol L4	0.27	0.50		
Ol H0	0.46	2.75	0.31	0.754
Ol H4	0.21	1.27		
C 0	0.46	0.45	0.52	0.602
Ol L0	0.15	0.58		
C 0	0.46	0.45	0.10	0.917
Ol H0	0.46	2.75		
C 4	0.27	0.39	0.10	0.917
Ol L4	0.27	0.50		
C 4	0.27	0.39	0.73	0.465
Ol H4	0.21	1.27		

C 0: control zero time, C 4: control 4 wks, Ol H0: olive oil high dose zero time, Ol H4: olive oil high dose 4 wks, Ol L0: olive oil low dose zero time, Ol L4: olive oil low dose 4 wks.Cal.Z is Calculated Z.; *Significant level is at ($p<0.05$).; Variable unit for BAP is in ng/mL.

Revealed the lowest MMP-2 value, while Ol L0 group showed the highest MMP-2 value. The highest BAP value was found in Ol H0 group, whereas the lowest BAP value was for C4 group. The highest TRAP5b value was found in C0 group, whereas the lowest TRAP5b value was found in OLl0 group.Regarding MMP-2, the present study was conducted in the aim to search for a natural inhibitor of MMP-2 activity during orthodontic retention period. The reasons behind choosing MMP-2 as biomarker in the current study because MMP-2 play an important role in the degradation of ECM, a process that takes place during processes involving CT degradation such as that accompanied histological reactions in PDL during the course of OT. Also due to the unique properties of MMP-2 in that it functions as both gelatinase and collagenase[(17)]. MMP-2 more specifically hydrolyze denatured collagens (gelatin), native types IV,V and XI collagens, and elastin, actsat regulation of vascularization, the inflammatory response and basement membrane breakdown [(25,26)]. All these processes are associated with histological reaction during the course of OT as the destruction of periodontium associated with OT period, is influenced by collagenase and gelatinase activity of MMP-2 [(27,28)]. The inflammatory reaction in periodontal tissue associated with OT is influenced by MMP-2 that plays a critical role in cell migration to site of inflammation [(29)].In this study, Ol L0 group showed significantly highest MMP-2 value than the remaining groups, a possible explanation to this high value may be due to limited period of Ol administration which is reflected on bioavailability of its active ingredients to the target tissues. On the contrary, OL H4 group exhibited a significantly lowest MMP-2 value comparing to the remaining study groups, suggesting beneficial role of Ol, when it is administered at high dose (15.4 ml/kg b.w.). This result came in accordance to, and support the studies

about the capability of natural compound inhibitors of enzymatic activity of MMP-2 of Gingras et al.[30], Friedman[31], Banerji et al.[32], Chen et al.[33], Kousidou et al.[34], Philips et al.[35], Balitaan et al.[36], Grzywnowics et al.[37], Ding et al.[38], Hassan and Daghestani [39], Noori-Daloii et al.[40], Yan et al.[41] and Park et al.[42]. However, these studies utilized diverse types of natural products rather than Ol and had proved inhibitory MMP-2 mechanisms by regulation of certain transcriptional factors and inhibition of the activated protein-1 (AP-1) system, downregulation MMP-2 mRNA and protein expression [40], that may cause the downregulation of MMP-14, enhancing MT1-MMP and the subsequent inhibition of MMP-2 [33].

Concerning BAP, to determine the effect of Ol on serum BAP, a well-known biomarker of osteoblastic differentiation activity and to assess whether Ol can promote the differentiation of osteoblasts, a serum Rabbit BAP ELISA detection kit was used. Measurement of bone turnover marker is increasingly recommended as a key component of therapy management involving bone tissue: to rapidly identify therapy responders and non-responders, to assess therapy efficacy and to determine the optimal therapy and dose of treatment [43]. Alkaline phosphatase has become the marker of choice when assessing the phenotype or developmental maturity of mineralized tissue cells [44]. Alkaline phosphatase is the most frequently used biochemical marker of osteoblastic bone formation [45]. Alkaline phosphatase can also be used as biochemical marker to determine osteoblast activity since it present on the fragments of plasma membranes of osteoblast cells [46]. Bone specific alkaline phosphatase was chosen as potential biomarker for bone formation during the first three months of OT [47], throughout OT [48-51], and as soon as the tooth movement stops [52]. According to Haima [43], BAP was demonstrated to be one of the most attractive bone turnover biomarker to date in comparison to other bone formation biomarkers as bone mineral density (BMD)which takes longer time to develop any detectable and significant changes in bone tissue. The enzyme BAP is present in the osteoblast membrane, participates in the synthesis and mineralization of the bone matrix, and is one of the most widely used markers of osteogenic differentiation [53]. Its expression may increase up to 27 times during the process of osteogenic differentiation [54]. Other cause for choosing alkaline phosphatase (ALP) is the fact that in both tissues, bone and calcifying cartilage, ALP is expressed early in development, and is soon observed on the cell surface and in matrix vesicles. Later in the developmental program, while other genes (e.g. osteocalcin) are upregulated, ALP expression declines. Clearly, ALP must function in the initial phases of the process [44].

In the current study, the results demonstrated no significant differences in all of the BAP values for group comparisons in statistical analysis. However, the highest plasma BAP, was found in the Ol H0 group, indicating an increased osteoblastic differentiation. The increase of ALP activity during the process of osteogenic differentiation found in Ol H0 group suggested successful osteogenic differentiation [55,56]. The present result came in agreement with Campos et al. [57] who found that Ol enhances intestinal absorption of calcium and Claassen et al.[58] who revealed that Ol is an excellent source of gamma linolenic acid, which had been shown to reduce the excretion of calcium, inhibit bone resorption and influence markers of bone turnover while at the same time increased the calcium content in the bone, and Trichopoulou et al.[59] who emphasized that Ol improves bone health due to its high content of MUFA, which reported to increase BMD. Additionally, the current result is supported by Puel et al.[60] study who found that Ol oleuropein prevented bone loss in ovariectomized (OVX) rats with 0.15 gm oleuropein/kg for 3 months by increasing metaphyseal and total femoral BMD in comparison to remaining study groups. However, their study showed that, oleuropein and Ol consumption had no effect on plasma osteocalcin concentrations (marker of bone formation) or on urinary deoxypyridinoline excretion (marker of bone resorption).

The result of the present study, as well, came in agreement to the study of Puel et al.[61] who assessed the dose-dependent bone-sparing effect of oleuropein on experimentally induced bone loss in OVX rats by reduction of bone loss, increasing BMD of the total femur metaphyseal and diaphyseal subregions and improvement of inflammatory biomarkers. Puel et al.[62] found that a high Ol polyphenol diet prevented bone loss in animals, which was suggested to be due to the antioxidant and anti-inflammatory effects of olive polyphenols, including hydroxytyrosol. Furthermore the present result was supported by Puel et al.[12], who proved that the daily consumption for 84 days of tyrosol and hydroxytyrosol, phenolic compounds of Ol, improved bone loss in experimental OVX rats by enhancing total, metaphyseal, and diaphyseal BMD and increasing bone formation, that attributed to their antioxidant properties.

The findings of Saleh and Saleh [14] study agreed with the current result. Saleh and Saleh determined protective effects of Ol supplementation against osteoporosis in OVX-female rats for 12 wks, 4 wks before ovariectomy and 8 wks after. In the Ol-OVX rats plasma levels of calcium, phosphorus, ALP, malondialdehyde, and nitrates were markedly increased as compared to the OVX-group and significant increase in the cortical bone thickness and the trabecular bone thickness of the tibia.

In addition, the current result was supported by Santiago-Mora et al. [13] who showed that oleuropein at concentration of 10-6 to 10-4 M, inhibited adipocyte differentiation and enhanced differentiation of mesenchymal crest cells into osteoblasts. In addition, the gene expression of osteoblastogenesis markers, runt-related transcription factor II, osterix, collagen type I, ALP, and osteocalcin, were higher in osteoblast induced oleuropein-treated cells.

The finding of our study, moreover, came in harmony with that of Hagiwara et al.[63]. They used oleuropein, hydroxytyrosol and tyrosol, (polyphenols in Ol), and cultured osteoblasts and osteoclasts to reveal their effects on bone loss in OVX mice. Only at concentration up to 10 μM. oleuropein and hydroxytyrosol at 10 to 100 μM, they had no effect on the production of type I collagen and the activity of ALP in cultured cells, but they stimulated the deposition of calcium in a dose-dependent manner. In contrast, oleuropein at 10 to 100 μM and hydroxytyrosol at 50 to 100 μM inhibited the formation of multinucleated osteoclasts in a dose-dependent manner. Furthermore, both compounds suppressed the bone loss of trabecular bone in femurs of OVX mice, while hydroxytyrosol attenuated H2O2 levels in cultured cells.

The current result was also supported by Ferna´ndez-Real et al.[64] findings on protective effects of virgin Ol on bone and reduction of age-related bone mass loss and bone strength. They examined the longitudinal effects of a Mediterranean diet enriched with virgin Ol on circulating forms of osteocalcin and bone formation markers (undercarboxylated osteocalcin, C-telopeptide of type I collagen, and procollagen I N-terminal propeptide concentrations) in elderly men.

Furthermore, results of the present study came in harmony with that of Liu et al.[65] study who found that extra virgin Ol significantly increased BMD and decreased phosphatase, ALP, interlukin-6 (IL-6), malonyldialdehyde, and nitrate levels, nevertheless they used female rats and extra virgin Ol that was given by oral gavage at a dose of 1 mL/100 gm weight on a daily basis for 12 consecutive wks.

Furthermore, the current result was supported by that of Tagliaferri et al.[66] who found that Ol improves BMD. They used refined or virgin Ol fortified with vitamin D3 for 30 days in OVX mice, bone remodeling (serum N-terminal propeptide of type I procollagen) as marker of bone formation and serum (collagen type 1 cross-linked C-telopeptide) as bone resorption marker and BMD. The lowest plasma BAP, although non-significant, was found in the Ol L0 group. This was in agreement with Haima [43] who stated that BAP levels decrease following anti-resorptive therapy Ol in a dose-dependent manner and this reduced BAP level indicating transitions of the osteoblast to an osteocyte [67] signifying advanced phases of AB turnover [68, 69].

Despite the agreement of the present result about the effect of Ol on BAP with the results of above mentioned studies, however, some of those studies utilized pure polyphenolic compound(s) of Ol rather than Ol, different bone formation biomarkers other than BAP, other in vitro and in vivo experimental models or even human subjects, different natural product concentrations and administration routes, different detection technique and various observational periods.

For serum TRAP5b values, in the present study, no significant difference was found for most of the values in group comparison regarding TRAP5b. Serum TRAP5b is probably the most reliable marker of bone resorption available [70]. Serum TRAP5b is derived exclusively from bone-resorbing osteoclasts. Contrary to most other commonly used bone markers, TRAP5b does not accumulate in blood circulation during renal or hepatic failure, has a low diurnal variability and is not affected by feeding. TRAP5b describes the number of osteoclasts in addition to their activity, and appears to be a highly specific and sensitive marker of bone resorption [71]. The biological and analytical variability of TRAP5b was lower than those of the other markers, making TRAP5b more reliable and allowing a smaller decrease to be significant. Determination of TRAP5b levels in blood samples would allow frequent, inexpensive and quantitative evaluation to evaluate disease progression as well as treatment effectiveness [70].

Because secreted TRAP5b is an accurate marker of osteoclast number, it can be conveniently used to replace histomorphometric determination of osteoclast number in animal models and microscopic counting of osteoclasts in in vitro bone cell cultures. The clinical performance of TRAP5b for treatment monitoring is among the best of known bone turnover markers [70,71].

The use of TRAP as bone resorption biomarker was in concordance to Takimoto and Mori [72],Keeling et al.[73], King and Keeling [74], Insoft et al.[75], Rody et al.[76], Noxon et al.[77], Ariffin et al. [78], Abdul Wahab et al.[79], Bhosale et al.[80], Abu Kasim et al.[81], Abdul Wahab et al.[50] and Abdul Wahab et al.[51] observation of TRAP activity accompanied with OT. Although those studies utilized salivary or gingival cervicular fluid TRAP rather than serum TRAP as a bone resorption biomarker, used various experimental models or even human subjects, used various orthodontic appliances with varying applied forces for different observational periods in comparison to the present study.

The Biological Explanation Behind Behavior of Olive Oil

In the present study the causes behind the obtained results was suggested to be due to anti-inflammatory and anti-oxidant effects of Ol polyphenols. The mechanism behind anti-inflammatory behavior of Ol polyphenols [10,12,62] may

be related to their ability to modulate the production of pro-inflammatory molecules as (IL-1β), tumor necrosis factor-alpha (TNF-α), Macrophage/ monocyte colony-stimulating factor (GM-CSF), cyclo-oxygenase-1 (COX-1), COX-2 and prostaglandins (PGs)(similar to that seen with NSAIDs) that are catalytic products of COX-2 [11,82] which play important roles for regulating MMPs, in particular, PGE2 [11] which has been reported to up-regulate MMP-2 [83] which is a vital enzyme degrading ECM proteins and also thought to induce bone cell proliferation, differentiation and migration [84]. Interlukin-1β, IL-6 and TNF-1αcan upregulates MMP-2 synthesis [85], especially IL-1, which directly stimulates osteoclast differentiation and function [86] via up-regulating the expression of receptor activator of nuclear factor ligand (RANKL) and/or down-regulating osteoprotegerin (OPG) in osteoblasts or stromal cells [87]. Olive oil polyphenols also module nitrous oxide (NO) production [88]. Nitrous oxide is known to be a potent stimulator of osteoclasts differentiation through its activation of the RANKL [89].

Also Ol phenolics have anti-oxidative actions [11,90]. This anti-oxidative actions of Ol phenols may be related to their ability to inhibit oxidative reactions that are involved in the beginning and progression of many human diseases via regulation of enzymatic activities [91].

Olive oil phenolics are able to react with reactive oxygen species (ROS) and chelate ROS producing metal ions, both of which allow for decreased oxidative DNA damage [92]. Nitric oxide reacts with free radicals, thereby producing high damaging peroxynitrite. Peroxynitrite can directly oxidize (Low Density Lipoprotein) LDLs resulting in irreversible damage to cell membranes. Olive oil phenolics causes scavenging of free radicals, therefore can no longer react with NO, resulting in less damage [93]. Nitric oxide interestingly can be viewed as radical itself and can directly be scavenged by flavonoids [94]. Free radicals can activate transcription factors that generate proinflammatory cytokines [94].

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