




RESEARCH ARTICLE

REVISED Effect of olive oil administration on the level of transforming growth factor β 1 during orthodontic tooth movement in old and young guinea pigs [version 2; peer review: 2 approved]

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Abstract

Background: Orthodontic tooth movement occurs due to continuous pressure on the teeth, causing the remodeling of the alveolar bone. The tissue will respond to bone growth factors, such as transforming growth factor β 1 (TGF- β 1), fibroblasts, and bone morphogenetic proteins, for new bone formation. The remodeling process is balanced in young adults, but there is an imbalance in older individuals due to decreased differentiation, activity, and life span of osteoblasts, and increased osteoclasts. Olive oil contains lots of antioxidants and can slow down the aging process. This study aims to study the differences in TGF- β 1 levels between old and young guinea pigs, and the difference between olive oil administration on TGF- β 1 levels in old and young guinea pigs with orthodontic tooth movement.

Methods: 12 guinea pigs divided into 4 groups: young guinea pigs (4-5 months) not given olive oil; young guinea pigs given olive oil; old guinea pigs (30-31 months) given olive oil; old guinea pigs given olive oil. The teeth were moved using an open coil spring mounted on the brackets on both lower incisors. Gingival sulcus fluid samples were taken on days 0, 7 and 14 of the movement of the teeth. TGF- β 1 levels were analyzed using ELISA.

Results: Three-way ANOVA and post hoc statistical tests showed that TGF- β 1 levels in young guinea pigs were significantly higher than old guinea pigs on days 0, 7 and 14 ($p < 0.05$). TGF- β 1 levels in both young and old guinea pigs who were given olive oil was significantly higher than those not given olive oil on days 0, 7 and 14 ($p < 0.05$).







Conclusions: TGF- β 1 levels in the younger age guinea pigs were higher than the older age, and olive oil could increase TGF- β 1 levels in the older age guinea pigs.



Keywords

olive oil, transforming growth factor β , orthodontic tooth movement

Open Peer Review

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REVISED Amendments from Version 1

This new version includes some minor changes in the background, discussion and detail of method sections.

Any further responses from the reviewers can be found at the end of the article

Introduction

Orthodontic treatment aims to correct malposition, malocclusion, and malrelation of dentoskeletal abnormality to get harmony, balance in aesthetics, face and head structure. Orthodontic tooth movement is a result of the remodeling of periodontal ligaments and alveolar bone¹. Bone remodeling during orthodontic tooth movement is described as a continuous and balanced process². The process of bone remodeling is regulated by osteoblasts and osteoclasts³. Osteoclasts in pressure areas will resorb the alveolar bone so that tooth movement occurs, and this phenomenon will be balanced by osteoblasts in the tension area⁴. Transforming growth factor- β 1 (TGF- β 1) is known as a multifunctional cytokine that plays an important role in bone formation. Moreover, TGF- β 1 activity increases with bone formation⁵.

The need for orthodontic treatment in adulthood increases with age, but the balance of bone resorption and remodeling processes decreases with increasing age⁶. Therefore, cellular activity in periodontal tissues of young and old individuals needs to be further studied. This may greatly influence the success of orthodontic treatment⁷. The process of aging is caused by free radicals, hormones, genetics, lifestyle, diet, pollution, and socioeconomic conditions. If these causative factors can be avoided, then the aging process can be prevented, slowed down, and may even be inhibited⁸.

Food ingredients that contain antioxidants can inhibit the oxidation process in the aging process. The oxidation process can increase intracellular reactive oxygen activity so that it can inhibit bone remodeling⁹. Olive oil is a food ingredient that rich of antioxidants and is often consumed by the public. Olive oil which extracted from fresh olives have been shown to slow the aging process¹⁰. According to Liu *et al.*, olive oil acts effectively as a substitute for estrogen to prevent bone loss¹¹. Melguizo-Rodríguez *et al.*, proved the phenolic compound in virgin olive oil extracts can increase the proliferation and differentiation of osteoblasts. Phenolic compounds (caffeic acid, ferulic acid, coumaric acid, apigenin, and luteolin) in olive oil extracts can increase TGF- β 1 and its receptor¹².

Therefore, olive oil is expected to reduce osteoclastogenesis so as to reduce tooth movement orthodontics, especially for cases to prevent loss of anchorage, periodontitis and tooth retention. The purpose of this study is to study the differences in TGF- β 1 levels between old and young ages, using guinea pig models, and the effect of olive oil administration on TGF- β 1 levels in old and young guinea pigs with orthodontic tooth movement. The guinea pig was chosen as an animal model as it has been previously used to study the effect of medications on orthodontic

tooth movement and has the potential to simulate the response of human tissue¹³.

Methods

Ethical clearance

Ethical clearance was approved by the Research Ethics Committee of the Faculty of Dentistry, University Gadjah Mada (UGM), Yogyakarta, Indonesia (00144/KKEP/FGK-UGM/EC/2019). "Animal Research: Reporting of *in vivo* Experiments" (ARRIVE) was used for reporting this study. During orthodontic appliance installation and gingival crevicular fluid collection, all the animals were anesthetized. We also used non-invasive techniques to move the teeth (using minimal 35 gr force, which is acceptable for guinea pig), and housing conditions that provided a comfortable and safe environment. All these procedures were done to ameliorate any suffering of animals used in this study.

Animals

We used 12 male guinea pigs (*Cavia porcellus*) (purchased from a commercial supplier, Kaliurang, Yogyakarta, Indonesia), which were chosen utilizing simple random sampling into four groups (n=3/group): young guinea pigs that were not administered olive oil (young guinea pig-control); young guinea pigs that were administered olive oil; old guinea pigs that were not administered olive oil (old guinea pig-control); old guinea pigs administered olive oil. Young guinea pigs were 4–5 months old and weighed approximately 300–400 grams, whilst old guinea pigs were 30–31 months old and weighed approximately 800–1000 grams. Sample size for the groups (n=3) was obtained utilizing Lemeshow's formula¹⁴. A sample size of three animals in each group would give more than 80% power to detect significant differences with 0.45 effect size and at a significance level of $\alpha = 0.05$. Sample groups were selected by means of simple random number sampling. Each animal was assigned a tag number, the blind-folded researcher then picks numbered tags from the hat.

All the animals were maintained for a week before the experiment began on an individual polycarbonate cage (30 cm \times 20 cm \times 20 cm) with a 12-h light/dark cycle at a steady temperature of 25°C and humidity of 50% for acclimatization to compensate for their various origins. All animals were fed a standard pellet diet (expanded pellets; Stepfield, UK) with tap water *ad libitum*.

Calculation of olive oil dosage and administration

We used extra virgin olive oil (Bertolli®, USA). Olive oil consumed by humans per day is 30–50 grams so the conversion of a human dose (70 kg) to guinea pig (400 grams) is 0.031. The dose was converted from human olive oil dose (weight, 70 kg) to a guinea pig dose (weight, 400g): $0.031 \times 30 \text{ ml} = 0.93 \text{ ml}$ ¹⁵. Based on body weight, the dose of olive oil given to young guinea pigs (300–400 g) was 0.7 ml and for old guinea pigs (800–1000 g) was 1.86 ml. Olive oil treatment groups (see below) were given olive oil daily from the beginning of the installation of orthodontic devices until day 14, while control groups received mock oil. Administration of oil was carried out orally with injection syringes that have been cut off (Figure 1) and were given at 9am every morning.



Figure 1. Administration of olive oil to a guinea pig.

Orthodontic installation

Guinea pigs were first anesthetized with ketamine (160095, Kepro™, Netherlands; 35mg/kgBW) and xylazine (160096, Xyla™, Netherlands; 5mg/kgBW) intramuscularly on the thigh, then a separator was placed between the two lower incisors (Figure 2). Next, the teeth were cleaned with rubber cups and pumice (22257, Kerr, USA) and an incisivus Roth bracket 0.022" (American Orthodontics, USA) was bonded on both lower guinea pig's incisor using an orthodontic adhesive (Transbond XT, 3M Unitek, USA), followed by light-curing for 40 s according to the manufacturer (10 s for each side: mesial, occlusal, gingival, and distal) using a quartz-tungsten-halogen (QTH) light-curing unit (Litex 680A, Dentamerica, USA) with a light intensity of 450 mW/cm². The wire arch mounted was 0.018" stainless steel wire arch, which was ligated using power O (American orthodontics, USA). Open coil spring NiTi (American Orthodontics, USA) was installed between the two brackets to move the teeth distally with a force of 0.35 N (Figure 3). The magnitude of the open coil spring force was measured using a tension gauge. The length of the stainless-steel wire was cut 4 mm longer than the open coil spring as a tolerance for orthodontic tooth movement.

Isolation of the gingival crevicular fluid

Gingival crevicular fluid (GCF) collection was performed on days 0, 7, and 14 after olive oil administration. Before removing GCF, the lower incisors of the guinea pig were cleaned with a cotton swab to remove supragingival plaque. Then the teeth were isolated with cotton wool and dried to exclude the remaining saliva. Next, a #15 paper point (Paper Points ISO 0.2, Dentsply, Germany) was inserted into the gingival sulcus of the mandibular incisor about 1 mm deep for 30 seconds to absorb all the GCF fluid and repeated thrice at an interval of 90 seconds to increase the volume of GCF fluid isolated (Figure 3). The paper point was then inserted into a 1.5 ml eppendorf tube containing 350 µl of saline solution. The eppendorf tube was centrifuged for 5 minutes at a speed of



Figure 2. Separator installation on a guinea pig to facilitate the installation of the bracket.

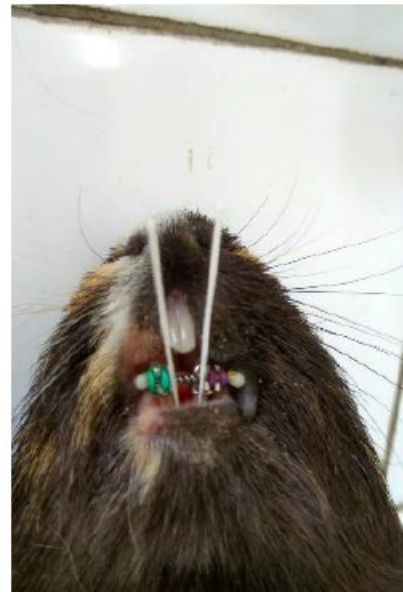


Figure 3. Installation of bracket and gingival crevicular fluid isolation.

2000 rpm to elucidate complete GCF components. Paper points were removed and the supernatant solution was stored at -80°C until required for analysis.

Detection TGF-β1 levels

Detection of TGF-β1 levels was carried out at the Molecular Biology Laboratory of the Faculty of Medicine UGM using ELISA

(enzyme-linked immunosorbent assay). The GCF was analyzed for TGF-β1 levels using ELISA kit reagent test (Cusabio, China), according to manufacturer protocols.

Data analysis

Three-way analysis of variance followed by post hoc statistical tests was used to analyse the difference among the groups and determine its significance. *P*-values <0.05 were considered statistically significant. All of the data were analyzed using SPSS software (Statistical Package for the Social Sciences, v22.0, USA).

Results

All the experimental procedures were well-tolerated. Furthermore, olive oil administration at the used dosage did not induce any general toxicity, including edema. The results of measurements of TGF-β1 levels of GCF before and after tooth movement can be seen in **Table 1**. The mean TGF-β1 levels

in the older age groups on days 0, 7 and 14 were higher than in the younger age groups, both in the olive oil groups and control groups. The mean TGF-β1 levels in the older and younger age groups increased on day 7 and decreased on day 14 (**Table 1**).

Figure 4 shows the administration of olive oil increases the average level of TGF-β1 in the young and old age groups. The mean TGF-β1 levels in the olive oil group were higher than the mean TGF-β1 in the group that were not given olive oil (control). The mean TGF-β1 levels in the old control and treatment groups and were higher than the young control and treatment on all days. Based on the time of observation, the highest TGF-β1 level was seen on day 7 and the lowest on day 0 in all groups. The highest TGF-β1 level was found in the young guinea pig group treated with olive oil at day 7 and the lowest TGF-β1 level was found in the old guinea pig group who were not given olive oil (young control) on day 0.

Table 1. TGF-β1 levels in gingival crevicular fluid among the two groups tested.

	Age	Group	Day 0	Day 7	Day 14
TGF-β1 (pg/ml)	Old	Control	23.19 ± 4.610	28.47 ± 4.648	23.51 ± 3.283
		Olive Oil	37.75 ± 4.052	49.92 ± 1.469	45.57 ± 3.330
	Young	Control	39.57 ± 3.754	53.84 ± 3.775	50.46 ± 4.732
		Olive Oil	50.56 ± 2.570	75.32 ± 1.515	66.14 ± 3.349

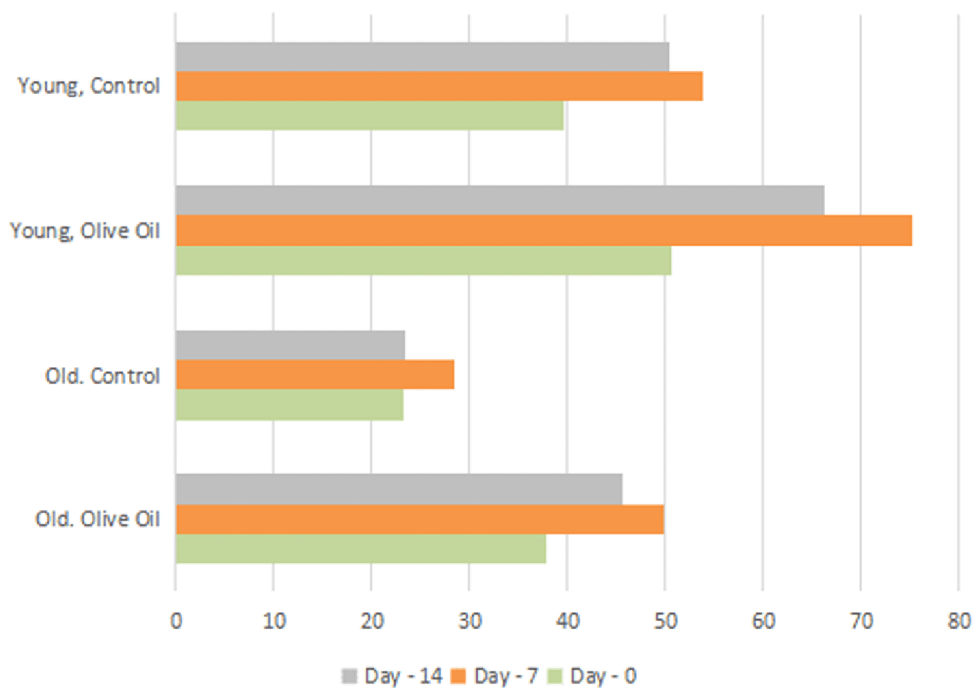


Figure 4. TGF-β1 levels (pg/ml) in gingival crevicular fluid of guinea pigs with tooth movement treated and untreated with olive oil.

Normality test (Saphiro-Wilk) and homogeneity test (Levene) showed normally distributed and homogeneous data. The research data were then analyzed using three-way ANOVA (Analysis of variance). The results of the three-way ANOVA (Table 2) showed that age, olive oil administration and observation time had a significant effect on TGF-β1 levels ($p < 0.05$). There was an interaction between administration of olive oil and observation time, and also between observation time and age,

but there was no interaction between administration of olive oil and age or administration of olive oil, observation time, and age ($p > 0.05$).

A post hoc test was performed after ANOVA to see groups that had significant differences ($p < 0.05$). The post hoc test results showed that most of the TGF-β1 activity of the old and young age groups who were given olive oil and not given olive oil on days 0, 7, and 14 showed significant differences (Table 3).

Table 2. Results of three-way ANOVA of TGF-β1 secretion in gingival crevicular fluid of old and young guinea pigs with teeth movement who were given olive oil and controls.

Variables	Significance
Olive oil	0.000*
Days of observation	0.000*
Age	0.000*
Olive oil- Days of observation	0.018*
Olive oil – Age	0.140
Days of observation – Age	0.002*
Olive oil - Days of observation – Age	0.461

Discussion

The process of bone remodeling is regulated by osteoblasts and osteoclasts. Osteoblasts and osteoclasts will appear 40–48 hours after being exposed with orthodontic forces¹⁶. Osteoclasts in depressed areas will absorb alveolar bone so that tooth movement occurs. This phenomenon will be offset by the apposition of osteoblasts in the tension area². The balance of bone resorption and remodeling processes decreases with increasing age, which greatly influences the success of orthodontic treatment^{6,7}. Olive oil has been shown to contain antioxidant compounds, namely phenolic compounds. Phenolic compounds are able to provide a strong protective effect against aging¹⁷⁻¹⁹. The results of the current data analysis showed that the average TGF-β1 level in GCF in the olive oil groups was higher than the mean TGF-β1

Table 3. Post hoc test results of mean TGF-β1 secretion in gingival crevicular fluid in old and young guinea pigs given olive oil and controls.

	OO D-0	OO D-7	OO D-14	OC D-0	OC D-7	OC D-14	YO D-0	YO D-7	YO D-14	YC D-0	YC D-7	YC D-14
OO D-0	-											
OO D-7	0.000*	-										
OO D-14	0.013*	0.150	-									
OC D-0	0.000*	0.000*	0.000*	-								
OC D-7	0.004*	0.000*	0.000	0.084	-							
OC D-14	0.000*	0.000*	0.000*	0.819	0.053	-						
YO D-0	0.000*	0.830	0.102	0.000*	0.000*	0.000*	-					
YO D-7	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	-				
YO D14	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.004*	-			
YC D-0	0.540	0.002*	0.051	0.000*	0.001*	0.000*	0.001*	0.000*	0.000*	-		
YC D-7	0.000*	0.193	0.009*	0.000*	0.000*	0.000*	0.273	0.000*	0.000*	0.000*	-	
YC D-14	0.000*	0.856	0.108	0.000*	0.000*	0.000*	0.974	0.000*	0.000*	0.001*	0.259	-

* = significantly different $p < 0.05$; OC = old, control; OO = Old, olive oil; YC = Young, control; YO = Young, olive oil.

in the groups that was not given olive oil (controls). The results of the three-way ANOVA (Table 2) showed that age, olive oil administration and observation time had a significant effect on TGF- β 1 levels ($p < 0.05$). TGF- β 1 is a multifunctional cytokine that modulates proliferation, growth, differentiation, adhesion and cell survival, but it also plays a role in the production of extracellular matrix proteins⁵. The application of orthodontic forces can activate TGF- β 1 transcription in these regions, thereby increasing TGF- β 1 levels in GCF¹². TGF- β 1 can recruit osteoblast precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix²⁰. During tooth movement, latent TGF- β 1 is activated in the periodontal tissue by plasmin, which is produced by retracted periodontal ligament cells and set in the cascade of plasminogen activator⁵. During bone formation, TGF- β 1 inhibits the recruitment of osteoclast precursor cells and directly inhibits osteoclast activity in bone resorption¹⁸. Moreover, TGF- β 1 in this area will stimulate osteoblastogenesis and new bone formation together with inhibition of osteoclastogenesis in this area^{5,19}. Previous study showed that TGF- β 1 can stimulate osteoclast apoptosis in the area. The effect of TGF β proapoptosis is mediated by several factors such as osteoblasts and stromal cells under TGF- β 1 control⁵.

The phenolic content in virgin olive oil extracts such as apigenin, luteolin, coumaric acid, ferulic acid and caffeic acid can increase the proliferation capacity and differentiation of osteoblasts¹². Inhibition of osteoclastogenesis is also seen in bone resorption markers, such as OPG (osteoprotegerin) and Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL)². The phenolic content in virgin olive oil extract can improve bone repair, and this is related to osteoblastogenesis and related factors, such as transcription factor 2 (RUNX-2), osterix (OSX), collagentype I (COL I) osteocalcin (OSC), and alkalinephosphatase (ALP). The phenolic compounds contained in virgin olive oil extract significantly increase the levels of TGF β -1 and its receptors, and TGF β -1 receptor expression is significantly increased in the presence of phenolic compounds¹².

Tooth movement in orthodontic treatment can be divided into three stages: initial phase, lag phase, and post lag phase. In the initial phase, the orthodontic force will produce 0.4-0.9 mm of tooth movement. This phase takes place within one week. Cellular reaction starts after the application of force, this is seen by the presence of osteoclasts, osteoblasts, osteoblast progenitors and inflammatory cells¹⁶. In the present study, TGF β -1 levels of in the young and old guinea pigs reached the maximum value on observation day 7 indicating that the movement of the teeth is in the initial phase. Decreased levels of TGF β -1 on day 14 indicate that tooth movement is in the lag phase. The lag phase is characterized by no or minimal movement of the teeth²¹.

Conclusion

Olive oil increases TGF β -1 levels in both young and old guinea pigs during orthodontic tooth movement. Further studies are necessary at clinical levels to confirm the efficacy and potency of olive oil in increasing bone remodeling in orthodontic patients, especially in older patients.

Data availability

Figshare: Picture1.jpg, <https://doi.org/10.6084/m9.figshare.10095101>²²

This project contains the following underlying data:

- Raw data.xlsx (raw data of TGF beta levels among two groups tested)
- Statistic TGF beta.docs (statistic data)
- Fig 1 (raw image used for Figure 1)
- Fig 2 (raw image used for Figure 2)
- Fig 3 (raw image used for Figure 3)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](#).

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<http://www.doi.org/10.6084/m9.figshare.10095101>

Open Peer Review

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Our initial questions were well addressed in the revised version, and we have no further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mechanisms of orthodontic tooth movement

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 May 2020

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We have no further comments to make.

Thank you.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Ervina Sofyanti : The findings that can influence the perception of urgency of integrated medical orthodontics approach; Denny Satria : molecular and cell biology in clinical cases that might require multidisciplinary study.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 09 March 2020

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The paper titled "Effect of olive oil administration on the level of transforming growth factor β 1 during orthodontic tooth movement in old and young guinea pigs" showed that TGF- β 1 levels in the younger age guinea pigs were higher than the older age, and olive oil could increase TGF- β 1 levels in the older age guinea pigs.

Here are a few questions:

- At first, several references of publications should be added in Discussion. For example, "TGF- β 1 can recruit osteoblasts precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix" and "During bone formation, TGF- β 1 inhibits the recruitment of osteoclast precursor cells and directly inhibits osteoclast activity in bone resorption." And please check full of the manuscript.

- In this paper, the number of guinea pigs used in the current experiments is 12. I want to know how did you calculate the required number size and why?
- In addition, as the animals in Olive oil treatment groups were treated with olive oil, were the animals in the control group treated with mock oil?
- Finally, please add more details about methods including Isolation of the GCF and Orthodontic installation in order for peers to reproduce your research.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mechanisms of orthodontic tooth movement

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 02 May 2020

Sri Suparwitri, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dear Dr **Mingyuan Du** and Dr **Hong He**

Thank you for your kind assistance in reviewing our manuscript and providing us with valuable advice for the manuscript and our future work. We have been working throughout the review. The following are our consolidated comments related to the review and some changes we have done based on the reviewer comments.

- At first, several references of publications should be added in Discussion. For example, "TGF- β 1 can recruit osteoblasts precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix" and "During bone formation, TGF- β 1 inhibits the recruitment of osteoclast precursor cells and directly inhibits osteoclast activity in bone resorption." And please check full of the manuscript.

We have edited the discussion section and added other references, Thank you for the punctiliously review. We have added this information in the present manuscript

- In this paper, the number of guinea pigs used in the current experiments is 12. I want to know how did you calculate the required number size and why?

We count it based on the Lemeshow's formula

$$(t-1)(n-1) \geq 15$$

$$(7-1)(n-1) \geq 15$$

$$n \geq 3,5$$

t represent a total group we determined on this study and n represent the sample. From those formula we have got 3.5 and base on animal restriction we decided to round numbered down into three samples for each group

- In addition, as the animals in Olive oil treatment groups were treated with olive oil, were the animals in the control group treated with mock oil?

Thank you for the punctiliously review. We have added this information in the present manuscript

- Finally, please add more details about methods including Isolation of the GCF and Orthodontic installation in order for peers to reproduce your research.

We have edited the methods section and added this information in the present manuscript.

We thank you again for the valuable insight.

Competing Interests: No competing interests were disclosed.

Reviewer Report 13 January 2020

<https://doi.org/10.5256/f1000research.23310.r57888>

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Ervina Sofyanti

Department of Orthodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

Denny Satria

Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

This title of manuscript has described the main question that addressed by this study. It is relevant and interesting topic in related to relapse of orthodontic treatment. It is necessary to have a scientific background that can show the common thread between previous researches and requirement of adequate orthodontic treatment (including relevant references for previous work and time citation) to understand the motivation and context of research by explaining the experimental approaches and reasons. Such as: The reason of using olive oil as sample is not too clear to prove why the authors using olive oil as sample to increase TGF-B expression in crevicular fluid, and the amount of crevicular fluid that is taken should be explained more detail.

Even though the text is clear and easy to be read, this manuscript may require more adequate well-written

English and alert to citations. Be careful in citation writing and bibliography. Then, I suggest to re-consider few references in introduction and discussion. Then explaining any steps taken to minimise the effects of subjective bias when allocating animals to treatment and describe who was blinded and when assessing results in this study.

“Some researchers show that TGF- β can stimulate osteoclast apoptosis in the area. The effect of TGF- β pro apoptosis is mediated by several factors such as osteoblasts and stromal cells under TGF- β 1 control⁵.” I recommend adding other references. Then I also recommend to re-consider the year of references and add some references in discussion.

Finally, we recommend to revise this manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dentistry especially related to orthodontics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 21 Jan 2020

Sri Suparwitri, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dear Dr. Ervina and Dr. Denny Satria

Thank you very much for your kind assistance in reviewing our manuscript. According to your minor suggestions, we have revised within the material and method section.

This title of the manuscript has described the main question addressed by this study. It is a relevant and interesting topic related to the relapse of orthodontic treatment. It is necessary to have a scientific background that can show the common thread between previous researches and the requirement of adequate orthodontic treatment (including relevant references for previous work

and time citation) to understand the motivation and context of research by explaining the experimental approaches and reasons. Such as The reason for using olive oil as the sample is not too clear to prove why the authors using olive oil as a sample to increase TGF-B expression in crevicular fluid, and the amount of crevicular fluid that is taken should be explained more detail.

Thank you for your kind suggestion. We already added some information about it in the present manuscript. “Food ingredients that contain antioxidants can inhibit the oxidation process in the aging process. The oxidation process can increase intracellular reactive oxygen activity so that it can inhibit bone remodeling. Olive oil is a food ingredient that contains a lot of antioxidants and is often consumed by the public⁷. Olive oil is extracted from fresh olives have been shown to slow the aging process⁸. According to Liu *et al.*, olive oil acts effectively as a substitute for estrogen to prevent bone loss⁹. According to Rodrigues *et al.*, The phenolic compound in virgin olive oil extracts can increase the proliferation and differentiation of osteoblasts. Phenolic compounds (caffeic acid, ferulic acid, coumaric acid, apigenin, and luteolin) in olive oil extracts can increase TGF- β 1 and its receptor⁷.”

Even though the text is clear and easy to be read, this manuscript may require more adequate well-written English and alert to citations. Be careful in citation writing and bibliography. Then, I suggest reconsidering a few references in the introduction and discussion. Then explaining any steps taken to minimise the effects of subjective bias when allocating animals to treatment and describe who was blinded and when assessing results in this study. We already added some information about it: “Sample groups were selected using simple random number sampling. Each animal was assigned a unique number, which was picked out of a box by a blindfolded researcher.”

“Some researchers show that TGF- β can stimulate osteoclast apoptosis in the area. The effect of TGF- β pro apoptosis is mediated by several factors such as osteoblasts and stromal cells under TGF- β 1 control⁵.” I recommend adding other references. Then I also recommend to re-consider the year of references and add some references in the discussion. We already added some information about it: “Previous study show that TGF- β 1 can stimulate osteoclast apoptosis in the area. The effect of TGF β proapoptosis is mediated by several factors such as osteoblasts and stromal cells under TGF β 1 control³.”

We also have added and revised our references in the present manuscript

Thank you for the review
Regards

Competing Interests: I have no competing interest to be reported

Author Response 02 May 2020

Sri Suparwitri, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dear Dr **Mingyuan Du** and Dr **Hong He**

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We thank you again for the valuable insight.

Competing Interests: No competing interests were disclosed.

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