Effect of olive oil administration on the level of transforming growth factor β1 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 β1 (TGF-β1), 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-β1 levels between old and young guinea pigs, and the difference between olive oil administration on TGF-β1 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-β1 levels were analyzed using ELISA.

Results: Three-way ANOVA and post hoc statistical tests showed that TGF-β1 levels in young guinea pigs were significantly higher than old guinea pigs on days 0, 7 and 14 (p<0.05). TGF-β1 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-β1 levels in the younger age guinea pigs were higher than the older age, and olive oil could increase TGF-β1 levels in the older age guinea pigs.

Keywords
olive oil, transforming growth factor β, orthodontic tooth movement
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 bone. Bone remodeling during orthodontic tooth movement is described as a continuous and balanced process. The process of bone remodeling is regulated by osteoblasts and osteoclasts. 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 area. Transforming growth factor-β1 (TGF-β1) is known as a multifunctional cytokine that plays an important role in bone formation. Moreover, TGF-β1 activity increases with bone formation.

The need for orthodontic treatment in adulthood increases with age, but the balance of bone resorption and remodeling processes decreases with increasing age. Therefore, cellular activity in periodontal tissues of young and old individuals needs to be further studied. This may greatly influence the success of orthodontic treatment. 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 inhibited.

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 rich of antioxidants and is often consumed by the public. Olive oil which extracted from fresh olives have been shown to slow the aging process. According to Liu et al., olive oil acts effectively as a substitute for estrogen to prevent bone loss.

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-β1 and its receptor.

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-β1 levels between old and young ages, using guinea pig models, and the effect of olive oil administration on TGF-β1 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 tissue.

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/FKG-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, Kalirang, 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 formula. A sample size of three animals in each group would give more than 80% power to detect significant differences with a 0.45 effect size and at a significance level of α = 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 × 20 cm × 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 × 30 ml = 0.93 ml. 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.
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 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 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.

| Age   | Group     | Day 0      | Day 7      | Day 14     |
|-------|-----------|------------|------------|------------|
| 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).

### 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 forces. 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 area. The balance of bone resorption and remodeling processes decreases with increasing age, which greatly influences the success of orthodontic treatment. Olive oil has been shown to contain antioxidant compounds, namely phenolic compounds. Phenolic compounds are able to provide a strong protective effect against aging.

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 level in controls; this suggests that olive oil may have a positive effect on bone remodeling, which could be beneficial in orthodontic treatment.

### 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        |

### 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.

| Variables                  | Significance |
|----------------------------|--------------|
| **Olive oil**              |              |
| OO                         |              |
| D-0                       |              |
| **OO D-7**                |              |
| **OO D-14**               |              |
| OC                         |              |
| D-0                       | 0.000*       |
| D-7                       | 0.013*       |
| D-14                      | 0.000*       |
| **OC D-0**                |              |
| **OC D-7**                |              |
| **OC D-14**               |              |
| YO                         |              |
| D-0                       | 0.000*       |
| D-7                       | 0.000*       |
| D-14                      | 0.000*       |
| **YO D-0**                |              |
| **YO D-7**                |              |
| **YO D-14**               |              |
| YC                         |              |
| D-0                       | 0.000*       |
| D-7                       | 0.000*       |
| D-14                      | 0.000*       |
| **YC D-0**                |              |
| **YC D-7**                |              |
| **YC D-14**               |              |

* = 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-β1 levels (p<0.05). TGF-β1 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 proteins. The application of orthodontic forces can activate TGF-β1 transcription in these regions, thereby increasing TGF-β1 levels in GCF. TGF-β1 can recruit osteoblasts precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix.

During tooth movement, latent TGF-β1 is activated in the periodontal tissue by plasmin, which is produced by retracted periodontal ligament cells and set in the cascade of plasminogen activator. During bone formation, TGF-β1 inhibits the recruitment of osteoclast precursor cells and directly inhibits osteoclast activity in bone resorption. Moreover, TGF-β1 in this area will stimulate osteoblastogenesis and new bone formation together with inhibition of osteoclastogenesis in this area. Previous study showed that TGF-β1 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-β1 control.

The phenolic content in virgin olive oil extracts such as apigenin, luteolin, coumaric acid, ferulic acid and afeic acid can increase the proliferation capacity and differentiation of osteoblasts. Inhibition of osteoclastogenesis is also seen in bone resorption markers, such as OPGL (osteoprotegerin) and Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL). 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), osteirx (OSX), collagentype I (COL I) osteocalcin (OSC), and alkalinephosphatase (ALP). The phenolic compounds contained in virgin olive oil extract significantly increase the levels of TGFβ-1 and its receptors, and TGFβ-1 receptor expression is significantly increased in the presence of phenolic compounds.

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 cells. In the present study, TGFβ-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 TGFβ-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 teeth.

Conclusion

Olive oil increases TGFβ-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

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).

References

1. Alhasyimi AA, Rosyida NF, Rihadni MS: Postorthodontic relapse prevention by administration of grape seed (vitis vinifera) extract containing cyanidine in rats. Eur J Dent. 2018; 13(4): 629–634. Published Abstract | Publisher Full Text | Free Full Text
2. Alhasyimi AA, Pudyani PP, Asmara W, et al.: Enhancement of post-orthodontic tooth stability by carbonated hydroxyapatite-incorporated advanced platelet-rich fibrin in rabbits. J Oral Sci. 2018; 60(1): 112–118. Published Abstract | Publisher Full Text
3. Seeman E: Bone modeling and remodeling. Crit Rev Eukaryot Gene Expr. 2009; 19(3): 219–33. Published Abstract | Publisher Full Text
4. Utogawa N, Takashashi N, Jimi E, et al.: Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/ RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone. 1995; 25(3): 517–23. Published Abstract | Publisher Full Text
5. Kobayashi Y, Hashimoto F, Miyamoto H, et al.: Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. J Bone Miner Res. 2002; 15(10): 1924–34. Published Abstract | Publisher Full Text
6. Ehrman JK, Gordan PM, Visich PS, et al.: Clinical Exercise Physiolog. Human Kinetics, Inc; USA, 2013: 448. Reference Source
7. Rody WJ Jr, Wijegunasinghe M, Wiltshire WA, et al.: Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. Angle Orthod. 2014; 84(1): 120–6. Published Abstract | Publisher Full Text
8. Kartiko BH, Siswanto FM: Hormon on anti aging medicine concept, J Virgin. 2015; 1(2): 108–22. Reference Source
9. Alhasyimi AA, Pudyani PS, Hafiz I: Effect of mangosteen peel extract as an antioxidant agent on the shear bond strength of orthodontic brackets bonded to bleached teeth. Dental J Orthod. 2018; 23(5): 58–64. Published Abstract | Publisher Full Text | Free Full Text
10. Berr C, Portet F, Carriere I, et al.: Olive oil and cognition: results from the three-city study. Dementia Geriatr Cogn Disord. 2009; 28(4): 357–364. PubMed Abstract | Publisher Full Text | Free Full Text

11. Liu H, Huang H, Li B, et al.: Olive oil in the prevention and treatment of osteoporosis after artificial menopause. Clin Interv Agin. 2014; 9: 2087–95. PubMed Abstract | Publisher Full Text | Free Full Text

12. Melgizo-Rodriguez L, Manzano-Moreno FJ, Illescas-Montes R, et al.: Bone Protective Effect of Extra-Virgin Olive Oil Phenolic Compounds by Modulating Osteoblast Gene Expression. Nutrients. 2019; 11(8): pii: E1722. PubMed Abstract | Publisher Full Text | Free Full Text

13. Suparwiti S, Pudyani PS, Haryana SM, et al.: Effects of soy isoflavone genistein on orthodontic tooth movement in guinea pigs. Dent J (Maj Ked Gigi). 2016; 49(3): 168–174. Publisher Full Text

14. Suhardjono: Percobaan hewan laboratorium. Gajah Mada University Press Yogyakarta, 1995; 207.

15. Tripoli E, Tabacchi G, Giammanco M, et al.: The phenolic compounds of olive oil; structure, biological activity and beneficial effects on human health. Nutr Res Rev. 2009; 18(1): 98–112. PubMed Abstract | Publisher Full Text

16. Masella RS, Meister M: Current concepts in the biology of orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2006; 129(4): 458–68. PubMed Abstract | Publisher Full Text

17. Keiter AM, Zierau O, Bernhardt R, et al.: Impact of a functionalized olive oil extract on the uterus and the bone in a model of postmenopausal osteoporosis. Eur J Nutr. 2014; 53(4): 1073–81. PubMed Abstract | Publisher Full Text

18. Tuck KL, Hayball PJ: Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem. 2002; 13(11): 636–44. PubMed Abstract | Publisher Full Text

19. Chen G, Deng C, Li YP: TGF-β1 and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci. 2012; 8(2): 272–87. PubMed Abstract | Publisher Full Text | Free Full Text

20. Indriasari V, Suparwiti S, Christnawati C, et al.: Different effects of soybean isoflavone genistein on transforming growth factor levels during orthodontic tooth movement among young and old rabbits [version 1; peer review: 1 approved]. F1000Res. 2019; 8: 2074. Publisher Full Text

21. Alhasyimi AA, Pudyani PS, Asmara W, et al.: Effect of carbonated hydroxyapatite incorporated advanced platelet rich fibrin intrasulcular injection on the alkaline phosphatase level during orthodontic relapse. AIP Conf Proc. 2018; 1933: 030006. Publisher Full Text

22. Suparwiti S: Picture1.jpg. figshare. Dataset, 2019. http://www.doi.org/10.6084/m9.figshare.1009510
Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 12 June 2020

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✔ Mingyuan Du
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Hong He
The State Key Laboratory Breeding Base of Basic Science of Stomatology (HubeiMOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, China

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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✔ Denny Satria
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Ervina Sofyanti
Department of Orthodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

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.
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-β1 can recruit osteoblasts precursors in the area of bone formation and stimulate osteoblasts to differentiate to produce bone matrix” and “During bone formation, TGF-β1 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

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**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-β1 control. 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-β1 and its receptor.”

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 proapoptosis is mediated by several factors such as osteoblasts and stromal cells under TGF-β1 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-β1 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 β1 control 3.

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 Suparwiti, 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-β1 can recruit osteoblasts precursors in the area of bone formation and stimulate
osteoblasts to differentiate to produce bone matrix” and “During bone formation, TGF-β1 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

$$\frac{(t-1)(n-1)}{15} \geq 3.5$$

$$\frac{(7-1)(n-1)}{15} \geq 3.5$$

n ≥ 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.