

Effects of Robusta coffee (*Coffea canephora*) brewing on levels of RANKL and TGF- β 1 in orthodontic tooth movement

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ABSTRACT

Background: Orthodontic tooth movement will be followed by periodontal ligament and alveolar bone remodeling. Orthodontic mechanical force (OMF) will be distributed through the teeth to periodontal ligament and alveolar bone and then will generate local pressure resulting in bone resorption and tension areas that will form new bone. Robusta coffee contains caffeine, chlorogenic acid and caffeic acid. Caffeine may increase osteoclastogenesis, and caffeic acid has antioxidant effects that may reduce oxidative stress in osteoblasts. **Purpose:** This study conducted to analyze the effect Robusta coffee steeping on levels of RANKL and TGF- β 1 in orthodontic tooth movement. **Method:** 16 male rats were divided into 2 groups. Group C: rats given OMF, Group T: given OMF and coffee brew at 20 mg/ 100 g BW. OMF in rats was conducted by applying ligature wire on the molar-1 (M-1) and both incisivus of right maxilla. Subsequently, M-1 of right maxilla was moved to mesial with a Niti closed coil spring. Observations were made on days 15 and 22 by taking the GCF by putting paper point on the gingival sulcus of mesio- and disto-palatal areas of M-1 of right maxilla to determine the levels of RANKL and TGF- β 1 using ELISA method. **Result:** The administration of coffee brew was effective to increase levels of RANKL and TGF- β 1 in the compression and tension areas ($p < 0.05$). RANKL levels in compression area were higher than in the tension area ($p < 0.05$), while the levels of TGF- β 1 in the tension area were higher than in the compression area ($p < 0.05$). **Conclusion:** The administration of coffee brew was effective to increase the levels of RANKL and TGF- β , therefore it might improve alveolar bone remodeling process.

Keywords: RANKL; TGF- β 1; Robusta coffee; orthodontic tooth movement; alveolar bone remodeling

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INTRODUCTION

The prevalence of malocclusion in Indonesia is still very high, approximately about 80% of the population. Malocclusion, consequently, is considered as the biggest dental and oral health problem. This condition is triggered by low dental care awareness and bad habits in society, such as sucking thumb or something else. Since the number and severity level of malocclusion will continually increase, so malocclusion must be prevented or treated.¹

Orthodontic treatment aims to adjust the position of teeth to the right tooth curve. Thus, chewing function efficiency, face harmony, oral health, dentofacial aesthetics,

and tooth position stability can be improved. Orthodontic treatment usually takes 2-3 years.²

Orthodontic tooth movement, will be followed by remodeling of alveolar bone and periodontal ligament.³ Orthodontic mechanical force will be distributed from the teeth to the periodontal ligament and the alveolar bone, resulting in bone resorption at pressure site and new bone formation at tension site during tooth movement.⁴

Application of orthodontic mechanical force on teeth is marked with inflammation activating macrophages, then releasing cytokines and growth factors.⁵ Those growth factors are receptor activator of nuclear factor κ B ligand (RANKL) and transforming growth factor β (TGF- β 1).⁶

RANKL is a regulator of bone remodeling during the orthodontic movement process.⁴

RANKL is expressed on osteoblasts and stromal cells as a respond to Parathyroid hormone (PTH) and stimulation triggered by active 1,25-dihydroxyvitamin D (1,25 Vit D3).⁷ RANKL binds to receptor activator of nuclear factor $\kappa\beta$ (RANK) on osteoclast precursors, triggering osteoclast differentiation and proliferation. As a result, osteoclasts then become active. Next, the active osteoclast will trigger bone resorption.⁸ On the other hand, osteoblasts also express Osteoprotegerin (OPG) as a receptor inhibiting RANKL-RANK interaction, resulting in prevention of osteoclastogenesis. Pressure force will mechanically induce osteoclastogenesis in vitro through an increase in RANKL expression and a decrease in OPG expression.⁹

The ratio of RANKL and OPG expressions is necessary to determine in inflammation inducing bone resorption. Bone resorption will occur if RANKL expression is higher than OPG expression. In contrary, bone formation will occur if OPG expression is higher than RANKL expression.¹⁰ There is also TGF- β 1 as a growth factor also considered as a periodontal homeostasis biomarker, promoting cell migration, cell differentiation, cell proliferation, as well as extracellular matrix synthesis. TGF- β 1 is also known as osteogenic protein, needed in bone mineralization.¹¹

Many efforts have been undertaken to accelerate orthodontic movement, such as medicines, surgical methods, as well as physical and mechanical stimulation methods.¹² One of materials used in those efforts are coffee. Coffee has recently been a popular drink consumed in the world. One of kinds of coffee consumed is Robusta coffee. Robusta coffee contains certain substance, known as Caffeine (1, 3, 7 trimetilxantin).¹³ Robusta coffee also contains chlorogenic acid and caffeic acid generating antioxidant effects.¹⁴ A research on rats given orthodontic mechanical force shows that the administration of caffeine at a high dose (10 mg/ 100 g BB) on them can improve osteoclasts and bone resorption at tension site on day 15.¹⁵ Caffeine increases osteoclastogenesis by improving RANKL.¹⁶ Caffeic acid has antioxidant effects that can reduce oxidative stress on osteoblasts.¹⁷ Another research also illustrates that chlorogenic acid promotes osteogenesis on human adipose tissue derived from mesenchymal stem cells (hAMSCs), indicated by an increase in bone mineralization.¹⁸

Therefore, this research aimed to analyze the effects of Robusta coffee brew on RANKL and TGF- β 1 levels during orthodontic tooth movement. The results of this research then were expected to reveal whether coffee could be used as a therapy for accelerating bone remodeling process and orthodontic tooth movement or not. As a result, orthodontic treatment could be conducted more easily, cheaper, and faster since coffee is easy to obtain and relatively cheap with minimal side effects.

MATERIALS AND METHOD

This research was a laboratory experimental study conducted on sixteen (16) healthy male rats (Sprague Dawley) aged 3-4 months and weighed 250-300 grams. Those rats were selected since they had complete dental structure as well as good oral cavity and periodontal tissue conditions. Those rats were divided randomly into two groups, namely control group (C), given orthodontic mechanical force and 2 ml of distilled water, and treatment group (T) given orthodontic mechanical force and drip of coffee brew at a concentration of 20 mg/ 100 g BW (equivalent to a cup of coffee for an adult man), dissolved into 2 ml of distilled water.

The administration of orthodontic mechanical force was conducted after those rats were anesthezing with ketamine. A ligature wire with a diameter of 0.20 mm was installed from their molar-1 (M-1) on the upper right jaw (UJ) to their two insivus. M-1 RA was moved into mesial by using tension gauge to generate a force of 10 g/ cm² with a nickel titanium orthodontic closed coil spring sized 6 mm length.¹⁹ Observation was conducted on days 15 and 22 to take gingival crevicular fluid (GCF) by putting paper point on mesio and disto-palatal areas of M-1 UJ for 30 seconds, and then put it into eppendorf tube.²⁰ RANKL and TGF- β 1 levels then were measured by using ELISA method. Installation of the closed coil spring on those rats can be seen in Figure 1.

Data obtained were analyzed using Student't-test, paired t-test, and Wilcoxon signed ranks test at a confidence level of 95% ($\alpha=0.05$). This research was approved by the research ethics committee of the Faculty of Dental Medicine, Universitas Airlangga with a letter no. 18/KKEPK.FKG/ II/ 2015.

RESULTS

The results of the research indicated that there were some effects of coffee brew on RANKL and TGF- β 1 levels



Figure 1 Installation of closed coil spring on the rats.

as shown in Table 1, Table 2, Table, 3 and Table 4. Table 1 shows the mean and standard deviations of RANKL levels at the pressure and tension sites on days 15 and 22. The results of the Wilcoxon signed ranks test on the pressure site and T test on the tension site on day 15 indicated that the RANKL levels in the treatment group were significantly higher than those in the control group ($p < 0.05$). Similarly, the results of T test on the pressure and tension sites on day 22 showed the RANKL levels in group T were significantly greater than in group C ($p < 0.05$). Those RANKL levels in groups C and T indicated that the pressure sites were larger

than the tension sites on day 15, but it was not statistically significant ($p > 0.05$). Meanwhile, the RANKL levels in groups C and T on day 22 indicated the pressure sites were significantly different from the tension sites ($p < 0.05$).

Table 2 illustrates the results of paired t-test on group C and Wilcoxon Signed Ranks test on group T. The results showed that the RANKL levels at the pressure sites of the research groups significantly decreased on day 22 compared to those on day 15, but it was not statistically significant ($p > 0.05$). On the other hand, the RANKL levels at the tension areas of the research groups decreased significantly on day 22 compared to those on day 15 ($p < 0.05$).

Table 1. Mean and standard deviation of RANKL levels at the pressure and tension sites in between the research groups

Groups	n	RANKL (pg/ ml) (Mean ± Standard Deviation)					
		On day -15			On day -22		
		Pressure	Tension	p	Pressure	Tension	p
C	8	17.30 ± 5.93	15.33 ± 4.40	0.514**	10.95 ± 4.16	5.98 ± 1.71	0.014*
T	8	41.82 ± 4.22	40.50 ± 3.85	0.484**	38.91 ± 4.95	32.72 ± 6.07	0.026*
		0.000*	0.000*		0.000*	0.000*	

Note: *: significantly different; **: insignificantly different

Table 2. Results of the difference test on RANKL levels at the pressure and tension sites between on day 15 and on day 22 in each group research

Group	n	RANKL (pg/ ml) (Mean ± Standard Deviation)					
		Pressure			Tension		
		On day-15	On day -22	p	On day-15	On day-22	p
K	8	17.30 ± 5.93	10.95 ± 4.16	0.101**	15.33 ± 4.40	5.98 ± 1.71	0.002*
P	8	41.82 ± 4.22	38.91 ± 4.95	0.208**	40.50 ± 3.85	32.72 ± 6.07	0.014*

Note: *: significantly different; **: insignificantly different

Table 3. Mean and standard deviations of TGF-β1 levels at the pressure and tension sites in between the research groups

Group	n	TGF-β1 Mean ± Standard Deviation)					
		On day-15			On day-22		
		Pressure	Tension	P	Pressure	Tension	p
C	8	3.83 ± 0.70	4.11 ± 0.65	0.271**	3.59 ± 0.91	3.76 ± 0.49	0.542**
T	8	24.26 ± 2.52	32.46 ± 4.95	0.006*	12.09 ± 1.54	15.75 ± 2.39	0.001*
T		0.000*	0.000*		0.000*	0.000*	

Note: *: significantly different; **: insignificantly different

Table 4. Results of the difference test on TGF-β1 levels at the pressure and traction areas between on day 15 and on day 22 in each group research

Group	n	TGF-β1 (pg/ ml) (Mean ± Standard Deviation)					
		Pressure			Traction		
		On day-15	On day-22	P	On day-15	On day-22	p
C	8	3.83 ± 0.70	3.59 ± 0.91	0.577**	4.11 ± 0.65	3.76 ± 0.49	0.313**
T	8	24.26 ± 2.52	12.09 ± 1.54	0.000*	32.46 ± 4.95	15.75 ± 2.39	0.000*

Note: *: significantly different; **: insignificantly different

Table 3 shows the mean and standard deviations of TGF- β 1 levels at the pressure and tension sites on days 15 and 22. The results of the T test on the pressure and tension sites on day 15 indicated that the TGF- β 1 levels in the treatment group were significantly higher than those in the control group ($p < 0.05$). The TGF- β 1 levels in group C indicated that the tension site was insignificantly larger than the pressure site on both days 15 and 22 ($p > 0.05$). Meanwhile, the TGF- β 1 levels in group T indicated that the tension site was significantly larger than the pressure site on both days 15 and 22 ($p < 0.05$).

Table 4 illustrates the results of paired t-test on group C and group T. The results showed that the TGF- β 1 levels at both of the pressure and tension sites in group C decreased on day 22 compared to those on day 15, but it was not statistically significant ($p > 0.05$). Meanwhile, TGF- β 1 levels at both of the pressure and tension areas in group T significantly decreased on day 22 compared to those on day 15.

DISCUSSION

The results of this research showed that the administration of coffee brew triggered an increase in RANKL levels at pressure and tension sites on days 15 and 22. This is due to caffeine contained in coffee binding to adenosine receptors and modulating several other receptors, including glucocorticoid receptor, insulin, estrogen, androgen, vitamin D, cannabinoid, glutamate, and adrenergic receptors, expressed in osteoblasts or osteoprogenitor cells which have important functions during osteoblast differentiation.²¹ An in vitro research shows that caffeine at a low concentration can also trigger cyclooxygenase-2 (COX-2)/ Prostaglandin E2 (PGE2), which then activate RANKL levels on osteoblasts, resulting in increased osteoclast formation, as well as reduce OPG expression on osteoblasts.²² Meanwhile, an in vivo research illustrates that caffeine can reduce bone mineral density (BMD) in rats and increase osteoclastogenesis. Previous research also shows that caffeine can improve an osteoclastogenic ability of periodontal ligament cells under stress, and increase tooth movement through PGE2-RANKL.¹⁶ Thus, a decrease in OPG expression may probably be caused by an increase in proinflammatory cytokine triggered by orthodontic pressure, will inhibit OPG expression.⁶

Results of this research also indicated the increased levels of RANKL at the pressure sites after the administration of coffee brew was larger than at the tension sites, especially on day 22. This is consistent with a research showing that the application of orthodontic force on pressure site can trigger osteoblasts to generate more RANKL expression, resulting in enhancement of osteoclastogenesis and then improvement of bone resorption.^{23,24}

In RANKL levels on day 22 decreased compared to those on day 15 either after the administration of coffee brew or not. This is because the strength of the mechanical

orthodontic force decreased on day 22, therefore osteoblast activity also decreased. The decreased levels of RANKL then could inhibit osteoclastogenesis and bone remodeling. As a result, it can be said that the administration of coffee brew can effectively increase the levels of RANKL on day 15.

The increased levels of TGF- β 1 at the pressure and tension sites on days 15 and 22 in this research is due to caffeic acid, phenolic acid classified into non acids phenolic flavonoids, contained in coffee, that can give an antioxidant effect in reducing oxidative stress on osteoblasts.¹⁷ Several in vitro and in vivo researches on experimental animals also show that oxidative stress can reduce the rate of bone formation by decreasing osteoblast differentiation and survival. A report even shows that reactive oxygen species (ROS) can activate osteoclasts, resulting in an increase in bone resorption. In other words, antioxidant activity is important in stimulating osteoblastic activity through specific receptors to support bone growth.²⁵

On day 22, the results of the research showed that the levels of TGF- β 1 at the pressure and tension sites after the administration of coffee brew significantly decreased compared to those on day 15. It is due to the reduced orthodontic pressure, so bone formation also decreased. The results of this research also indicated that TGF- β 1 levels were greater at the tension sites than those at the pressure sites since the tension sites always requires more bone formation than the pressure sites. TGF- β -1, produced by various cells, including osteoblasts and fibroblasts stimulated mechanically, has a highly osteogenic properties that can enhance osteoblast activity and inhibit osteoclast activity.²⁶ A research also shows that TGF- β 1 can be associated with tissue remodeling in periodontal ligament during orthodontic tooth movement, and the mechanical loads of the tension strength can regulate TGF- β 1 expression in osteoblasts and periodontal ligament cells in vitro.⁹ It can be concluded that the administration of coffee brew can increase RANKL and TGF- β 1 levels in order to improve alveolar bone remodeling process.

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