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Research Report

Evaluation of orthodontic tooth movement by 3D micro-computed tomography (μ-CT) following caffeine administration

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ABSTRACT

Background: *The compressive strength of orthodontic tooth movement will be distributed throughout the periodontal ligament and alveolar bone, resulting in bone resorption on the pressure side and new bone formation on the tension side. Caffeine, a member of the methyl xanthine family, represents a widely-consumed psychoactive substance that can stimulate osteoclastogenesis through an increase in RANKL. A 3D Micro-Computed Tomography (μ-CT) x-ray device can be used to measure orthodontic tooth movement and changes in periodontal ligament width.* ***Purpose:*** *The purpose of this research was to analyze the effects of caffeine on the distal movement distance of two mandibular incisors using 3D μ-CT.* ***Methods:*** *This research constituted an experimental study with post test control group design. The research subjects (guinea pigs) were randomly divided into four groups. Of the two control groups created, one received two weeks of treatment and the other three weeks. The members of these two control groups were subjected to orthodontic movement but received no caffeine. Meanwhile, the other two groups were treatment groups whose members also received either two or three weeks of treatment. In these two treatment groups, the subjects were subjected to orthodontic movement and received a 6 mg/500 BM dose of caffeine. The orthodontic movement of the subjects was induced by installing a band matrix and orthodontic bracket on each mandibular incisor to move distally by means of an open coil spring. Observations were then conducted on days 15 and 22 with μ-CT x-rays to measure the distal movement distance of the two mandibular incisors and the width of the periodontal ligament.* ***Results:*** *The administration of caffeine increased the tooth movement on day 15 ($p<0.05$) and day 22 ($p<0.05$). The increase in the tooth movement on day 22 was greater than that on day 15 ($p<0.05$). The width of the periodontal ligament on the pressure side of the treatment groups experienced greater narrowing than that of the control groups ($p<0.05$). Meanwhile, the width of periodontal ligament on the tension side of the treatment groups widened more than that of the control groups ($p<0.05$).* ***Conclusion:*** *μ-CT x-ray can be used to evaluate the extent of orthodontic movement in addition to the width of the mandibular incisor periodontal ligament during orthodontic tooth movement. Moreover, it has been established that the administering of caffeine can improve orthodontic tooth movement.*

Keywords: *caffeine; micro-computed tomography; orthodontic tooth movement*

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INTRODUCTION

The success of orthodontic treatment depends on periodontal tissue health, oral hygiene and orthodontic strength.[1] The latter causes the periodontal tissue to be divided histologically into pressure and tension sides.[2] Bone resorption occurs in the periodontal ligament subject to pressure, while bone formation occurs in the periodontal ligament experiencing tension.[3] Osteoclast activities will increase on the pressure side, whereas osteoblasts will start to proliferate and extracellular matrix mineralization, resulting in bone remodeling, occurs in the tension side.[4]

Osteoclasts play a significant role in bone resorption[5], while the alveolar bone marrow plays a role in osteoclast formation during orthodontic tooth movement.[6] When orthodontic mechanical forces are applied, changes that

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occur to the bone are accepted by mechanoreceptors regulated by osteocytes. These will then stimulate osteoclast proliferation and differentiation.

The differentiation of osteoclasts is regulated by two important cytokines, namely; macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κβ ligand (RANKL). M-CSF is an important factor responsible for the survival and proliferation of osteoclast precursors which also triggers expression of receptor activator of nuclear factor-ββ (RANK) in osteoclast precursor cells to generate an efficient response to the RANKL-RANK signaling pathway.[6] The binding of RANKL-RANK receptors to osteoclast precursors subsequently stimulates osteoclast differentiation and proliferation rendering osteoclasts active. These active osteoclasts will then induce bone resorption.[7]

Previous research on male Wistar rats posited that RANKL expression on day 21 increases significantly followed by bone resorption caused by orthodontic tooth movement. In addition, the results of the polymerase chain reaction (PCR) examination featured in that research also indicated that RANKL increases significantly on the pressure side.[8]

Orthodontic treatment is of relatively lengthy duration. Consequently, numerous efforts have been made to accelerate it, including drugs, surgical methods and physical/mechanical stimulation methods. At present, no drug exists capable of safely accelerating orthodontic tooth movement.[9]

Caffeine, on the other hand, is an alkaloid compound (purine base) that is white and psychoactive. It is usually consumed in the forms of coffee, tea and carbonated drinks such as cola, but is also used as a central nervous system stimulant (in this case, diuretic) which accelerates metabolism. Moreover, consumption of caffeine is beneficial since it increases alertness, eliminates drowsiness and enhances mood.[10] Prior research conducted on mice indicated that during orthodontic tooth movement low doses of caffeine (2.5 mg/100 g BM) can increase the number of osteoclasts, while simultaneously accelerating bone resorption on the pressure side of the alveolar bone on day 14.[11]

A method has been developed to enable the viewing of three-dimensional (3D) images of dentoskeletal and craniofacial relationships before and after orthodontic treatment. A 3-dimensional picture can show the results of treatment on both hard and soft tissues, such as the teeth, face and bones, while also being used to determine the diagnosis, prognosis and treatment plan.[12]

Unfortunately, it is not possible to use histology, a scanning electron microscope (SEM), a transmission electron microscope (TEM) or 3-dimensional imaging as means of observing periodontal tissue responses to orthodontic tooth movement with any degree of precision. Consequently, 3D micro-computed tomography (μ-CT) x-ray method has been developed to observe tooth movement and periodontal ligament width during this process.[13] As a result, the research aims to observe and analyze the effect of caffeine on the distal movement distance of two mandibular incisors using the 3D μ-CT method.

MATERIALS AND METHODS

This research constituted an experimental study with posttest control group design. Ethical approval was granted by the Research Ethics Committee of the Faculty of Medicine, Universitas Jember, No: 1150/H25.1.11/KE/2017. The research was conducted at the Biomedical Laboratory of the Faculty of Dentistry, Universitas Jember and included the administering of treatment of subjects through to tissue sampling. The subjects consisted of 20 male guinea pigs aged 10-12 months and weighing 500 grams which were randomly divided into four groups of five members.[14] The tissue samples were observed and measured to detect any increase in tooth movement among the subjects at the Micro-CT Laboratory of the Faculty of Mathematics and Natural Science (FMIPA) at Institut Teknologi Bandung (ITB).

Of the four groups to which the subjects had been randomly assigned, one was a 2-week control group and another a 3-week control group. The members of these two control groups, received orthodontic movement and 3ml of distilled water. Meanwhile, the other two groups constituted those receiving 2 and 3 weeks of treatment respectively. The members the two treatment groups were subjected to orthodontic movement and received a daily 6 mg/500 BM dose of caffeine (equivalent to that contained in one cup of coffee containing 100 grams of coffee powder in 150ml of water) dissolved in 3ml of distilled water.

At the next stage, the subjects were anesthetized with ketamine and orthodontic movement was set by installing the band matrix and orthodontic bracket on each mandibular incisor to enable it to move distally using an open coil spring (Ortho-tech, America)[15] with a power of 0.0525 N or 52.5 grams (Figure 1). Observations were then made after the subjects had been sacrificed on Days 15 and 22 by extracting both their right and left mandibular incisors as well as periodic tissue and placing these in the fixative solution.

Observations were subsequently performed by scanning the periodontal tissue samples with X-ray devices using the 3D method μ-CT Bruker SkyScan 1173 High Energy Micro-CT at the Micro-CT Laboratory of the ITB Faculty of Mathematics and Natural Science (FMIPA). Scanning was performed at 65 kV and 30 μA with a 1mm-sized aluminum filter for 250 minutes. 20 samples were scanned at standard resolution, producing output in the form of a 560560 pixel-sized projection image in 16-bit TIFF format which was recorded using an average of ten frames to minimize the noise produced. This device uses a silent source method and a rotating object detector which, for the purposes of this scan, operated with a rotation hose of 0.4°

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and a total rotation of 240°. The spatial resolution of the resulting image was ~50 micrometers/pixel. The complete image in the form of a 3D map of object density was then obtained by reconstructing the projection image using the Feldamp backpropagation method. After the reconstruction process, 539 pieces with a 2D image were produced in an 8-bit bitmap image format. Initial processing in the form of repositioning objects in 3D space and analyzing objects in the form of long measurements using DataViewer software (Bruker Micro-CT, Belgium) were subsequently carried out.

Observations of sagittal pieces were conducted from the crown to the root of the mandibular incisors in order to perform two functions. Firstly, to quantify the increased tooth movement in the subjects by measuring the distance

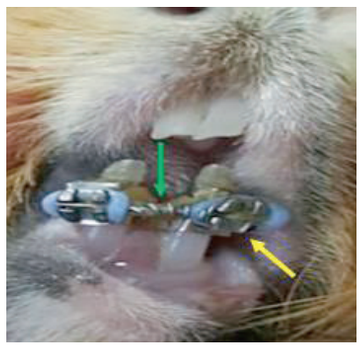

Figure 1. Installation of orthodontic devices in guinea pigs. The orthodontic bracket attached to the band matrix (yellow arrow) was affixed to the two right and left mandibular incisors of the guinea pigs, their two right and left mandibular incisors subsequently being moved distally using an open coil spring (green arrow).

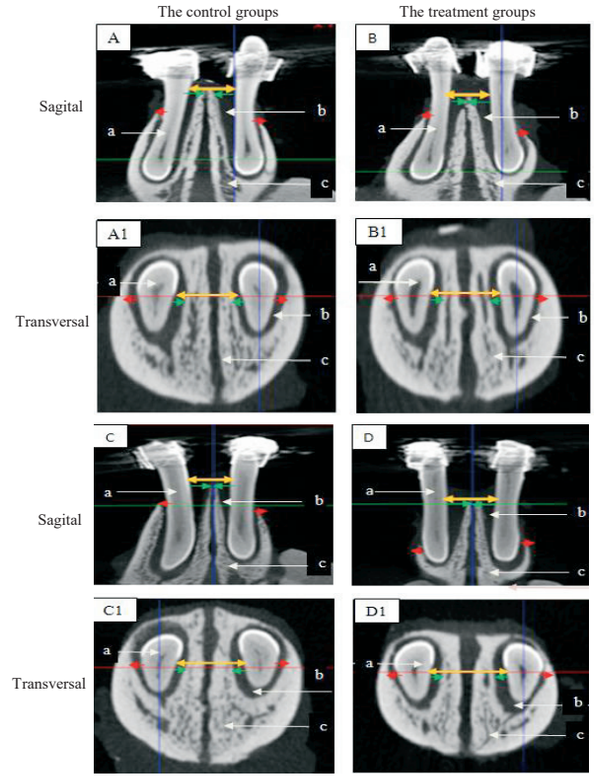

Figure 2. µ-CT Figures of the distal movements of the two mandibular incisors and the widths of periodontal ligaments on the sagittal pieces (A and B for 2 weeks; C and D for 3 weeks) and on transverse pieces (A1 and B1 for 2 weeks; C1 and D1 for 3 weeks); tooth root (a); periodontal ligament (b); alveolar bone (c); yellow arrow: the distal movement distance of incisor; green arrow: the width of the periodontal ligament in the apposition area; red arrow: the width of the periodontal ligament in the resorption area.

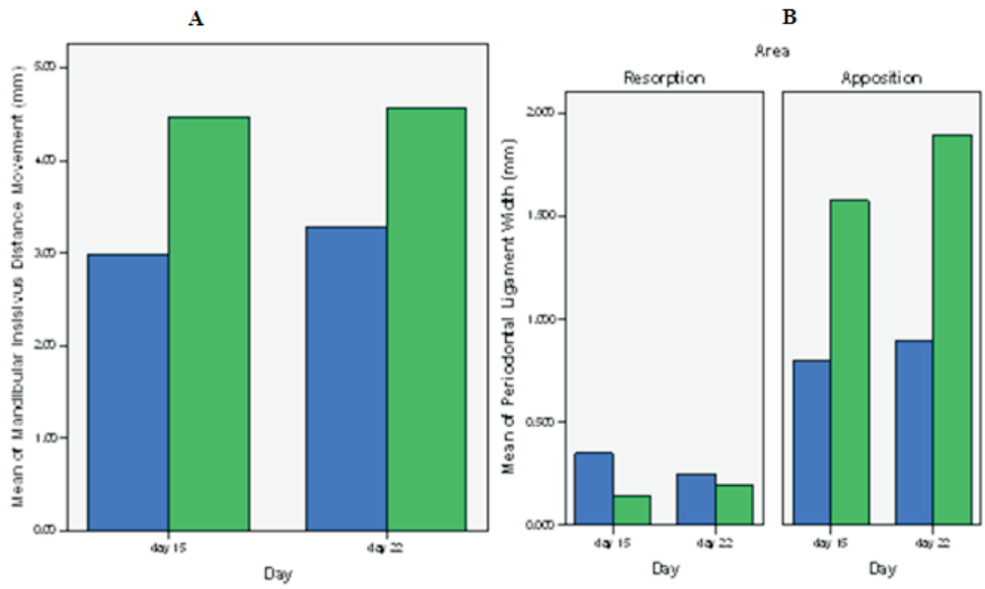

Figure 3. Histograms of distal movement distances for the two mandibular incisors (the width between the two mandibular incisors) (A) and the width of periodontal ligament in the resorption and apposition (B) areas in the research groups for two weeks and three weeks marked in green and the caffeine treatment group marked in blue.

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from the mesial section of the right mandibular incisor through the alveolar bone peak between the two teeth to the mesial section of the left mandibular incisor. Secondly, to measure the width of the periodontal ligament both in the mesial part of the left and right mandibular incisors representing the tension side or bone apposition area as well as in the distal part representing the pressure side or bone resorption area in the right and left first mandibular incisors (Figure 2). Thereafter, the mean width of the periodontal ligament on both pressure and tension sides of the two incisors was measured. The research data were analyzed using an independent t test with a confidence level of 95% (α=0.05).

Table 1. The mean and standard deviation of the distances or widths between the two mandibular incisors, as well as the results of a comparison test between the research groups on day 15 and day 22.

Groups	n	Tooth Movement (mm) (Mean ± Standard Deviation)		p
		Day-15	Day-22	
Control group	5	2.987±0.004	3.273±0.003	0.000*
Treatment group	5	4.472±0.002	4.572±0.002	0.000*
p		0.000*	0.000*	

Note: *Based on the independent t-test results

RESULTS

The results of this research into the effects of caffeine on the distal movement of mandibular incisors using the 3D μ-CT method are illustrated in Tables 1, 2, 3; as well as Figures 2 and 3. The distal movement of mandibular incisors was indicated by changes in the width or thickness of the periodontal ligaments in the cervical region measured from the mesial side of the right mandibular incisor through the alveolar bone to the mesial side of the left mandibular incisor (Figure 2 and 3) .

Table 1 shows the mean and standard deviation of the distal movements of two mandibular incisors in the treatment and control groups on days 15 and 22. The distal movements of two mandibular incisors in the treatment groups were greater than those in the control groups. Based on the t-test results, it is evident that significant differences in the distal movements of mandibular incisors existed between those groups on day 15 ($p<0.05$) and day 22 ($p<0.05$). This indicates that the administration of caffeine can elevate the distal movement of mandibular incisors to a greater extent than in cases where caffeine is not administered.

Table 2. The mean and standard deviation of the widths of periodontal ligaments in the resorption and apposition areas of each research group on day 15 and day 22.

Groups	n	Width of periodontal ligaments (mm) (Mean ± Standard Deviation)					
		Day-15			Day-22		
		Resorption	Apposition	p	Resorption	Apposition	p
Control group	5	0.349 ± 0.001	0.797 ± 0,001	0.000*	0.147 ± 0.001	0.897 ± 0.001	0.000*
Treatment group	5	0.147 ± 0.001	1.577 ± 0.001	0.000*	0.113 ± 0.003	0.180 ± 0.001	0.000*
p		0.000*	0.000*		0.000*	0.000*	

Note: *Based on the independent t-test results

Table 3. The results of the comparison test on the width of periodontal ligament in the resorption and apposition areas between day 15 and day 22 in each research group.

Groups	n	Width of periodontal ligaments (pg/ml) (Mean ± Standard Deviation)					
		Resorption			Apposition		
		Day-15	Day-22	p	Day-15	Day-22	p
Control group (-)	5	0.349± .001	0.147± .001	0.000*	0.797 ± .001	0.897± .001	0.000*
Treatment group (P)	5	0.147 ± .001	0.113± .003	0.000*	1.577±0.001	0.180± .001	0.000*

Note: *Based on the Independent T-Test results

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Table 2 depicts the mean and standard deviation of the periodontal ligament width of the resorption and apposition areas in each research group on days 15 and 22. Based on the t-test results, the width of the periodontal ligament in the resorption area in the treatment groups were smaller than those in the control groups on both days 15 ($p<0.05$) and 22 ($p<0.05$). Meanwhile, the widths of the periodontal ligament in the apposition areas of the treatment groups were greater than those in the control groups on both days 15 ($p<0.05$) and 22 ($p<0.05$). This means that the provision of caffeine can increase the distal movement of the mandibular incisor.

Table 3 shows that the results of the comparison test on periodontal ligament widths in the resorption and apposition areas in each research group on days 15 and 22. According to the t-test results, the width of the periodontal ligament in the resorption area on day 22 was smaller than that on day 15 ($p<0.05$). Meanwhile, the width of the periodontal ligament in the apposition area on day 22 was wider than that on day 15 ($p<0.05$). This means that the longer the duration of caffeine administration, the greater the distal movement of mandibular incisor (Figure 2).

DISCUSSION

The force of orthodontic pressure will be distributed through the teeth into the periodontal ligament and alveolar bone. This will result in bone resorption in the pressure side during tooth movement while, on the other hand, new bone formation will be triggered in the tension side.[16] Such movement caused by orthodontic treatment can actually cause sequential reactions involving periodontal tissue and alveolar bone, resulting in the release of various substances from dental tissues and surrounding structures. The initial response of the periodontal tissue to mechanical stress involves metabolic changes that enable tooth displacement. Minor changes in the thickness of the periodontal ligament occur one hour after the administration of orthodontic strength, while significant changes occur after a period of six hours.[17]

During orthodontic tooth movement, the tension side of the periodontal ligament will widen due to initial movement and fibroblast proliferation.[18] Complex molecular signals produce cellular responses to resorb alveolar bone with the result that the tooth moves. Thus, orthodontic tooth movement will be followed by alveolar bone and periodontal ligament remodeling processes.[7] A previous study of mice revealed that during molar teeth movement the presence of RANKL and RANK in periodontal tissues, in addition to mechanically stressed periodontal ligament cells, induces osteoclastogenesis through increased regulation of RANKL expression,[8] followed by bone resorption on the pressure side.[19] During orthodontic treatment, the application of optimal strength is important for adequate biological response in the periodontal system. However, the force applied during orthodontic treatment should not exceed capillary blood pressure (20-25g/cm)[7] since, if it does surpass that level, tissue necrosis can ensue.[16] Large forces can exert excessive pressure on the periodontal ligament and cause hyalinization which can, in turn, inhibit alveolar bone surface resorption.[20]

Another earlier study into the response of periodontal tissue after orthodontic tooth movement using TEM and SEM was carried out, but initial changes in the thickness of the periodontal ligament have yet to be identified. Contrastingly, in histological studies the thickness of periodontal ligaments is easily influenced by tissue preparation, such as decalcification and tissue dehydration. Moreover, the limited size of tissue sample preparation slides render it impossible to obtain an overall 3-dimensional picture of orthodontic tooth movement.[12,13]

3D μ-CT constitutes a new image examination with high-resolution and non-intrusive analysis techniques that have developed rapidly in recent years. Therefore, 3D μ-CT is expected to provide qualitative and quantitative data with three-dimensional images of specimens tested in order to learn about and better understand the breakdown of alveolar bone microstructure.[21,22] The 3Dμ-CT technique has also been employed to measure tooth movement and changes in the width of the periodontal ligament.[13] Previous research on periodontal tissue, especially in the trabecular bone microstructure, applies histological techniques capable of observing microstructure, but the parameters of alveolar bone microstructure remain challenging to obtain and describe.[23]

The results of evaluation using 3D μ-CT techniques on sagittal and transverse pieces obtained during this research showed that the provision of caffeine increased the distal movements of the two mandibular incisors on days 15 and 22 indicated by an increase in the intervening distance. The results also confirmed that the width of the periodontal ligament, indicated by the radiolucent area between the teeth and the alveolar bone on the pressure side, demonstrated narrowing resorption. In contrast, that on the tension side showed an apposition area that was increasing in width. In the caffeine treatment groups, the pressure side narrowed to a greater extent than that in the control groups, whereas the tension side of the caffeine treatment groups had greater periodontal ligament width. This indicates that tooth movements were more pronounced in the treatment groups supplied with caffeine.

The increasing distal movement of mandibular incisors is caused by caffeine generating osteoclastogenesis through an increase in RANKL.[24,25] The results of this research are in accordance with those of several previous investigations which posited that a low dose of caffeine can elevate osteoclasts and bone resorption on the pressure side of alveolar bone on Day 14.[11] Research conducted by Yamaguchi (2009), indicated that RANKL expression increases significantly followed by bone resorption caused by orthodontic tooth movement on day 21.[8]

RANKL, a member of the tumor receptor necrosis factor (TNF) family, mediates signals that lead to

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osteoclastogenesis.[26] RANKL plays a role in bone resorption and will interact with RANK on osteoclast precursors, thereby triggering osteoclast differentiation and proliferation resulting in osteoclasts becoming active and leading to bone resorption.[7,27] This will, in turn, cause orthodontic tooth movement[28] followed by remodeling of periodontal ligament and alveolar bone.[7]

Caffeine binds to adenosine receptors and modulates several other receptors including: glucocorticoid, insulin, estrogen, androgens, vitamin D, cannabinoids, glutamate and adrenergic receptors, all of which are expressed in osteoblasts or osteoprogenitor cells and have important functions during osteoblast differentiation.[29] Moreover, Vitamin D will promote greater transcription of RANKL and limit the production of osteoprotegerin (OPG).[30]

Previous research has argued that, while low caffeine concentration (0.005-0.01 mM) cannot affect cell survival and osteoblast differentiation from bone marrow mesenchymal stem cells (MSC), it can significantly increase both osteoclast differentiation from bone marrow hematopoietic stem cells (HSCs) and bone resorption activity. Such research has also indicated that lower caffeine concentration can increase RANKL protein expression in osteoblast cell cultures and reduce OPG expression in osteoblasts. Meanwhile, in vitro research has confirmed the findings of the aforementioned investigations that low caffeine concentration triggers cyclooxygenase (COX-2)/ prostaglandin (PG) E2 and increases RANKL in osteoblasts, thereby promoting osteoclast formation.[31]

The absence of mononuclear osteoclast precursors from the normal periodontal ligaments of mice and the presence of active osteoclasts there are caused by the pressure exerted by the orthodontic appliance. Mononuclear precursors in differentiated bone marrow will become mononuclear osteoclast precursors, before migrating to the periodontal ligaments on the pressure side and differentiating into active multinuclear osteoclasts. Multinuclear osteoclasts, subsequently, induce bone resorption.[32]

In addition, the orthodontic tooth movement on day 22, following the provision of caffeine, was significantly greater than that on day 15. It means that the longer the duration of caffeine provision, the greater the subsequent orthodontic tooth movement. As a result, caffeine is expected to be an alternative in accelerating orthodontic treatment. Finally, it can be concluded that 3D μ-CT method can observe changes in tooth movement and periodontal ligament width during orthodontic tooth movement both in the resorption and apposition areas. Furthermore, it is also known that caffeine provision can accelerate orthodontic tooth movement.

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