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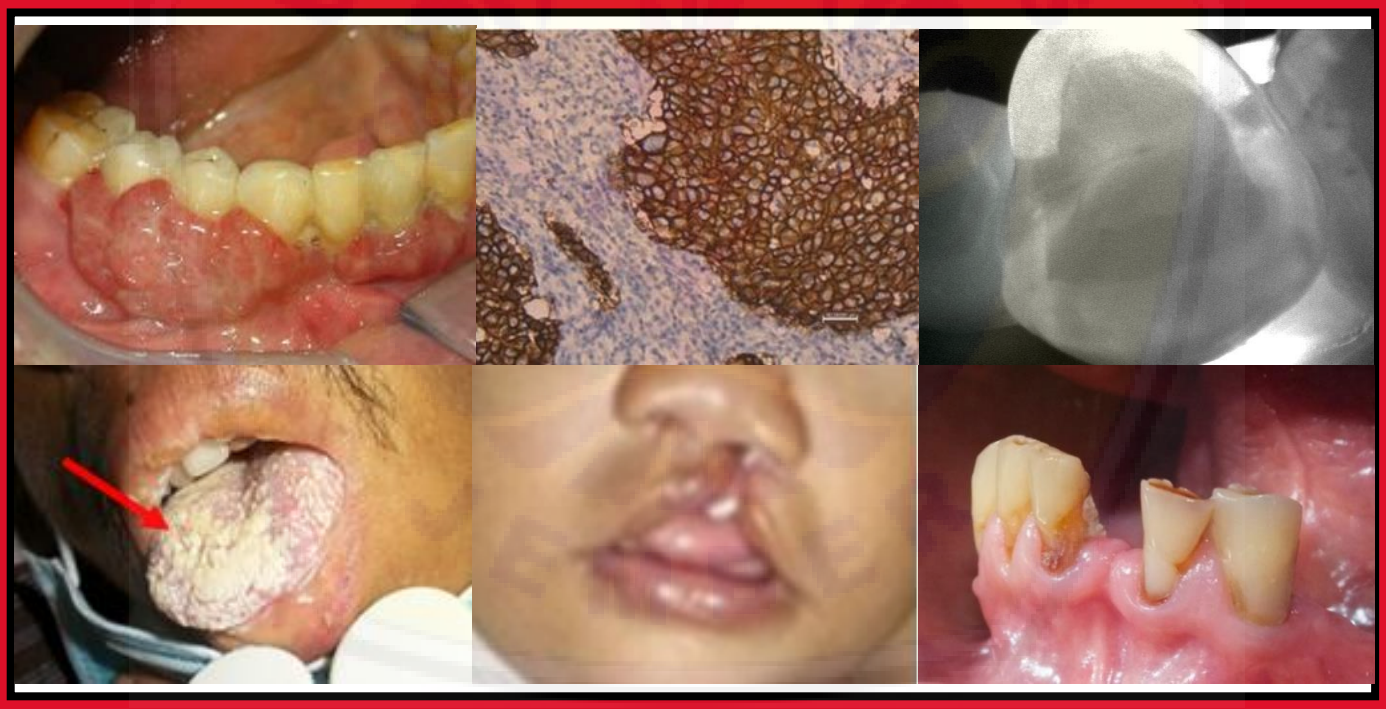

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NFATc1 and RUNX2 Expression on Orthodontic Tooth Movement Post Robusta Coffee Extract Administration

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Abstract

This study aimed at analyzing the expression of NFATc1 and RUNX2 on orthodontic tooth movement post administration of Robusta coffee extract.

A number of 14 wistar rats were divided into 2 groups, i.e. control group (C): rats applied with orthodontic mechanical force (OMF) and Treatment group (T): rats with OMF and Robusta coffee extract 20mg/100g BW. OMF for rats was applied by a ligature wire with a diameter of 0.20 mm on the maxillary right first molar and both maxillary incisors. Subsequently, the maxillary right first molar was moved to mesial using Niti closed coil spring. Observations were performed on day 15 with immunohistochemical examination to determine the expression of NFATc1 and RUNX2.

Robusta coffee extract increased NFATc1 and RUNX2 expression in compression and tension areas ($p < 0,05$). The expression of NFATc1 in the compression area was greater than that in the tension area ($p < 0.05$), whereas RUNX2 expression in the tension area was greater than that in the compression area ($p < 0.05$).

Robusta coffee extract enhances the expression of NFATc1 and RUNX2 on orthodontic tooth movement, thus it can be used as an alternative to accelerate orthodontic treatment.

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Introduction

Orthodontic treatment is commonly conducted using various appliances to treat jaw shape abnormalities and can improve occlusion to overcome aesthetic and functional problems. However, longer treatment periods have limited its clinical application. Thereby, tooth movement control and effective short time of treatment is an important clinical issue that should be addressed for better treatment [1.2].

Orthodontic tooth movement is a complex biological and mechanical process involving various molecules. The orthodontic force in the periodontal tissue activates osteoclasts and osteoblasts resulting in resorption and deposition of alveolar bone, and stimulates cell growth and collagen regeneration [3]. Orthodontic tooth movement depends on alveolar bone remodeling. The formation of osteoclasts is a prerequisite of alveolar bone remodeling. Under pressure stimulation, osteoclasts produce an osteoclastic reaction. It has been shown that tooth movement can be effectively regulated by controlling osteoclast differentiation and function[4]. Osteoblasts that function in bone formation are necessary to remodel the resorption region in the compression area and to form new bone in the compression area and tension area[5].

Nuclear Factor of Activated T-cells 1 (NFATc1) is the main regulator of the receptor activator of Nuclear Factor-κB Ligand (RANKL) which induces osteoclast differentiation and plays an important role in fusion and osteoclast activation through enhancement of osteoclast regulation of various genes responsible for osteoclast attachment, migration, acidification, inorganic degradation and bone organic matrix[7]. RANKL produced by osteoblasts further binds the Receptor activator of Nuclear Factor-κB (RANK) to the surface of osteoclast precursors and recruits adapter protein of tumor necrosis factor receptor-associated factor6 (TRAF6),

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which causes activation of Nuclear factor-kappa B (NF-kB) and transmits it to the nucleus. NF-kB increases the expression of c-Fos, and it interacts with NFATc1 to trigger transcription of the osteoclastogenic[8].

Runt-related transcription factor-2 (RUNX2) is a multifunctional transcription factor and is involved in the osteoblast cell differentiation process[9]. RUNX2 is a specific transcription factor of osteoblasts that activates and initiates differentiation of bone marrow mesenchyme cells into osteoblasts and regulates osteoblast maturation[10,11], and plays a key role in the process of mature osteoblasts[12]. During the movement of orthodontic teeth, the RUNX2 levels in the tension area are higher compared to that in the compression area[13].

Tooth movement on orthodontic treatment that takes a long time and is relatively high cost will become economic burden to the patient thus efforts to shorten the time of orthodontic treatment need to be conducted continuously. To accelerate the movement of the tooth on orthodontic treatment various attempts have been made, among others, using some drugs[14].

Various studies have been conducted recently to determine the effects of substances and medicines that have influence on orthodontic tooth movement i.e. medicines that show negative effects related to inhibition of orthodontic tooth movement e.g. osteoprotegerin and bisphosphonate (Pamidronat)[15,16], or medicines that show a positive effect i.e. acceleration of orthodontic tooth movement e.g. caffeine[17,18.] PGE2 injection may also increase the movement of the teeth[19], but it may lead to excessive resorption of bone and root surfaces[20], whereas the newest results of researches suggest that topical administration of PGE2 gel does not cause root resorption thus it may be considered for medication during orthodontic tooth movement[21].

Coffee is one of the popular beverages consumed among the public. Robusta coffee, among others, contains substances e.g. caffeine (1,3,7 trimetilxantin) [22], chlorogenic acid and caffeic acid, which have effect as an antioxidant [23], that can reduce oxidative stress on osteoblasts [24]. The results in rats have shown that administration of high doses of caffeine (10 mg / 100 g BW) increase the number of osteoclasts and bone resorption in the compression area on day 14[25].

The administration of caffeine 50mg/kg in pregnant rats have high osteogenic potential of osteoblasts characterized by increased expression of osteocalcin, osteopontin, sialoprotein, RUNX-2, alkaline phosphatase and collagen type 1 as well as the increase of synthesis of mineralized nodules[26]. Chlorogenic acid in coffee improves osteogenesis in human adipose tissue derived Mesenchymal stem (hAMSCs), indicated by mineralization increase, mRNA levels of alkaline phosphatase and RUNX2-2 transcription factor required for osteoblast differentiation[27].

This study was conducted to analyze the expression of NFATc1 in osteoclasts and RUNX2 in osteoblasts during orthodontic tooth movement post Robusta coffee extract.

Materials and methods

This laboratories experimental study was conducted using 14 male wistar rats aged 3-4 months and weighed 250-300, in good health, have complete tooth structure, oral conditions, and healthy periodontal tissue. Rats were randomly divided into 2 groups: control group (C): performed with orthodontic mechanical force and 2ml aquades, and Treatment group (T): performed with mechanical force of orthodontic and freeze dried extract of Robusta coffee of 20mg/100 g BB (equivalent to 1 cup of coffee for an adult person) dissolved in 2ml aquades. The mechanical force of orthodontics in rats was performed by anesthesia using ketamine. A ligature wire (3 M Unitek, Germany) with a diameter of 0.20 mm was applied in the maxillary right first molar and both maxillary incisors. Subsequently, the maxillary right first molar was moved to mesial using Tension Gauge (Ormco, USA) to produce 10 g/cm2 strength with Nickel Titanium Orthodontic closed coil spring (3 M Unitek, Germany) length 6 mm [28]. Observation was conducted by sacrificing the rats on day 15 and extracted the maxillary right first and second molars along with periodontal tissue. Immunohistochemical tests were performed to determine the expression of NFATc1 in osteoclasts and RUNX2 in osteoblasts. NFATc1 and RUNX2 expression. Observations were conducted using a microscope at 400x magnification. Data were analyzed using independent t-test, Mann-Whitney test, paired t-test and Wilcoxon signed ranks-test with 95% of

trust level (α = 0,05). This study has been approved by the ethical research committee of the Faculty of Medicine, Jember University, Number: 1150/H.25.1.11/KE/2017.

Results

The results of research on Robusta coffee extract effect on NFATc1 expression on osteoclasts and RUNX2 in osteoblasts are shown in Table 1 and Table 2 (see the appendix). Immunohistochemical results of the expression of NFATc1 and RUNX2 in the compression and tension areas are shown in Figures 1 and 2.

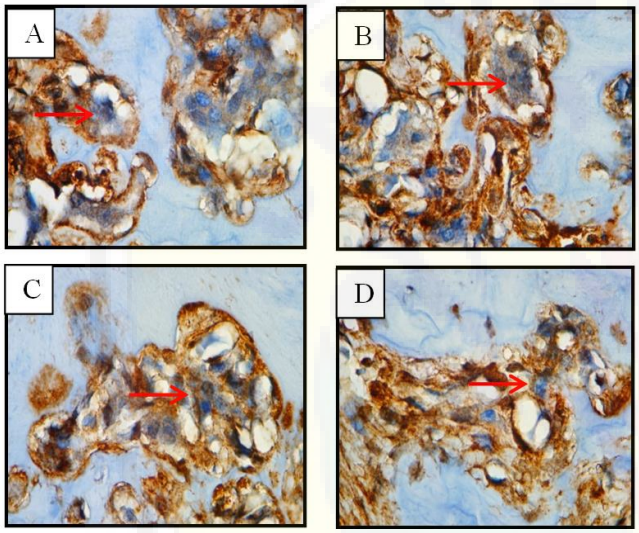

Figure 1. The expression of NFATc1 on osteoclasts is indicated by arrows: in Control group of Compression area (A), Control group of Tension area (B), Treatment group of Compression area (C) and Treatment group of Tension area (D), (Imunohistochemical, 400x magnification).

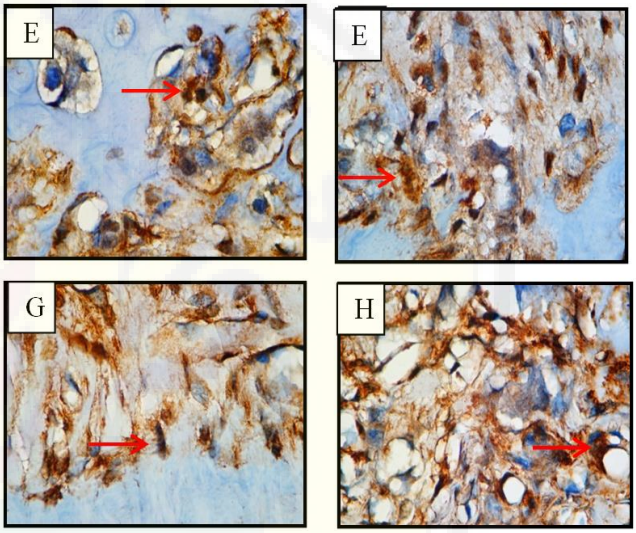

Figure 2. RUNX2 expression on the osteoblasts is indicated by arrows: in the Control group of compression area (E), Control group of Tension area (F), the Treatment group of Compression area (G) and the Treatment group of Tension area (H), (Immunohistochemistry, 400x magnification).

Groups	n	NFATc1 (Mean ± Standard Deviation)		p
		Compression Area	Tension Area	
Control (C)	7	5,86 ± 1,87	6,43 ± 1,62	0,522
Treatment (T)	7	12,57 ± 1,72	7,00 ± 1.41	0,016
P		0,000	0,710	

Table 1. Mean ± Standard Deviation of NFATc1 Expression and results test of differentiation test among the research groups of Compression Area and Tension Area.
Notes: p < 0.05 = Significant, p > 0,05 = Not Significant.

Groups	n	RUNX2 (Mean ± Standard Deviation)		p
		Compression Area	Tension Area	
Control (C)	7	3,71 ± 1,38	3,71 ± 1,38	1,000
Treatment (T)	7	6,14 ± 1,77	12,71 ± 1,89	0,000
P		0,014	0,000	

Table 2. Mean ± Standard Deviation of RUNX2 expression and results test of differentiation test among the research groups of Compression Area and Tension Area.
Notes: p < 0.05 = Significant, p > 0,05 = Not Significant.

Table 1 illustrates the description of the data in the form of mean and standard deviation of NFATc1 expression on the osteoclast in the compression and tension areas. Tests based on paired t-test showed that the expression of NFATc1 in osteoclasts in group C at the tension area was greater than that in the compression area but not significant (p> 0.05), whereas based on Wilcoxon signed ranks-test in group T, NFATc1 expression on osteoclasts in the compression area was significantly greater than the tension area (p <0.05). Differentiation test results based on independent t-test of NFATc1 expression on osteoclasts between the study groups in the compression area showed a significant difference (p <0.05), whereas the Mann-Whitney test in tension area showed no significant difference (p < 0.05).

Table 2 illustrates the description of the data in the form of mean and standard deviation of RUNX2 expression on osteoblasts in the compression and tension areas. Tests based on paired t-test showed that RUNX2 expression in

osteoblasts in group C in the compression area was equal to that in tension area or no significant difference (p> 0,05), whereas based on paired t-test in group T, RUNX2 expression on osteoblasts in the tension region was significantly greater than that in the compression area (p <0.05). Differentiation test results based on independent t-test of RUNX2 expression between research groups in the compression and the tension areas showed significant differences (p <0.05).

Discussion

NFATc1 plays an important role in osteoclast fusion. The process through regulation of transmembrane-specific regulation of dendritic cell Protein (DC-STAMP) and isoform d2 of vacuolar ATPase Domain V0 (Atp6v0d2) [29]. There is evidence that NFATc1 also regulates the activation of osteoclasts and it has been shown that NFATc1 activation enhances osteoclast formation and activation *in vivo* [30].

An increase in the amount of NFATc1 expression in *Robusta* coffee extracts in both compression and tension areas is due to the caffeine content in coffee increasing RANKL [17,18]. Caffeine increases RANKL expression in osteoblasts and increases osteoclastogenesis by increasing cyclooxygenase-2 (COX-2)/Prostaglandin 2 (PGE2) [31]. RANKL binds to its receptor RANK, which recruits adapter molecules e.g. TRAF6. It activates NF-κB, which is important for early induction of NFATc1. NFATc1 is activated by calcium signaling and binds its own promoter, allowing switching on an autoregulatory loop. The activator protein (AP) -1 complex containing c-Fos is required for autoamplification of NFATc1 leading to strong induction of NFATc1. Finally, NFATc1 works with other transcription partners to activate the specific osteoclast gene[32].

The increased expression of NFATc1 in the ccompression area is greater than that in the tension area in accordance with previous research results indicating that the application of orthodontic forces in the compression area increases RANKL expression which further increases osteoclastogenesis[17,18], thus bone resorption also increases and subsequently lead to orthodontic tooth movement followed by remodeling of the alveolar bone and the periodontal ligament[33].

Osteoblasts are differentiated from mesenchymal precursors, and mature osteoblasts to form osteoid followed by bone mineralization process[34]. Osteoblastic differentiation in human alveolar bone involves an increase in the expression of Runx2/Core Binding Factor a-1 (Cbfa1) which is an essential component of the differentiation process[35].

RUNX2 induces differentiation multipotent mesenchymal cells into immature osteoblasts and triggers bone matrix gene expression including osteocalcin (OCN), alkaline phosphatase (ALP) and others, in the early stages of osteoblast differentiation[36].

Increased expression of RUNX2 in osteoblasts in the compression and tension areas in the administration of Robusta coffee extract is due to caffeine content in coffee increases RUNX2 expression[24]. Caffeine contained in coffee binds to adenosine receptors and modulates several other receptors including glucocorticoid, insulin, estrogen and androgen receptors, Vitamin D, cannabinoid, glutamate and adrenergic receptors, all of which are expressed in osteoblasts or progenitor cells and have an important function during osteoblast differentiation[37,38.39]. Previous research has shown that caffeine with a concentration of 0.1 mM in adipose-derived stem cells (ADSCs) and bone marrow stromal cells, enhances osteoblast differentiation through activation of RUNX2[40].

Coffee also contains Chlorogenic acid that can increase RUNX2 [27]. Caffeic acid in coffee i.e. a phenolic acids has effect as an antioxidant that can reduce oxidative stress in osteoblasts[24]. Antioxidant activity is important in stimulating osteoblastic activity through specific receptors[41], thus the expression of RUNX2 in osteoblasts also increased. The expression of RUNX2 in the tension area is greater than that in the compression area confirming the results of the previous study that the extract of Robusta coffee also showed that the amount of osteoblasts in the tension area is greater than that in the compression area[42].

Conclusions

It is concluded that the extract of Robusta coffee increases the expression of NFATc1 and RUNX2, thus it can be an alternative to improve the process of bone remodeling and accelerate the movement of orthodontic teeth.

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Declaration of Interest

The authors report no conflict of interest.

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