ORIGINAL ARTICLE

Effect coffee administration on alkaline phosphatase levels during relapse following orthodontic retention in rats: an experimental study

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ABSTRACT

Introduction: Maintaining teeth in their corrected positions following orthodontic treatment can be extremely challenging. Coffee is one substance whose effects on relapse have been extensively studied. The aim of study is to analyze the effect of coffee administration on relapse after orthodontic retention by evaluating alkaline phosphatase levels. **Methods:** This experimental laboratory study enrolled SpraqueDawley rats, with brackets applied using *Edgewise* slot 0.022" system and a nickel-titanium open coil spring fixed between the lower central incisors for 14 days. Based on Federer's formula, 36 *SpraqueDawley* rats were randomly divided by simple random sampling into four groups: Group 1 (no retention), Group 2(retention for 3 days), Group 3(retention for 7 days), and Group 4(retention for 2 weeks). The *Spraque-Dawley* rats in the four experimental groups were given aquadest, 50 mg, and 100 mg/kg coffee intake ad libitum. Orthodontic relapse was assessed by measuring changes in alkaline phosphatase levels between the lower central incisors (the relapse side). Changes in the distance between the central incisors during relapse were observed visually. Alkaline phosphatase levels, which serve as a predictor of recurrence, were determined using an enzyme-linked immunosorbent assay (ELISA). Data analysis was conducted with the *Two-way analysis of variance* (*ANOVA*) and a *post hoc Tukey test*. **Results:** Overall, we found that a longer retention period was associated with a slower rate of relapse and a shorter overall amount of relapse. In addition, inhibiting osteoclast formation using coffee also reduced orthodontic relapse. Alkaline phosphatase (ALP) and/or its biomarkers could serve as potential therapeutic targets in the prevention and treatment of orthodontic relapse, with changes observed at a value of 0.001. This demonstrates that coffee administration affects alkaline phosphatase levels during relapse after orthodontic retention. **Conclusion:** Coffee administration increased alkaline phosphatase levels during relapse after orthodontic retention in rats.

KEYWORDS

Alkaline, phosphatase levels, Coffee, Relapse

INTRODUCTION

The prevention of relapse can be achieved through the use of passive orthodontic retainers, which help maintain the teeth in their corrected position over an extended period, allowing the supporting structures to reorganize after orthodontic treatment is complete. Biological treatments that inhibit bone resorption and promote bone formation can also effectively reduce the risk of relapse. Managing the remodeling of alveolar bone following orthodontic tooth

movement is an essential approach in preventing relapse.[1] Caffeine (1,3,7-trimethylxanthine) is one of the most common central nervous system stimulants used in drinks, foods, and medications.[2] The impact of coffee consumption on bone metabolism, bone density, and healing has been studied extensively, tough the results remain conflicting.[3]

Relapse after orthodontic tooth movement occurs rapidly once the teeth are no longer subjected to orthodontic force.[4] Studies of orthodontic relapse indicate a tendency for higher numbers of osteoclasts to be associated with greater distances of tooth movement.[5] To prevent relapse, the activity of osteoclasts must be suppressed.

Increased osteoblast activity during bone formation is accompanied by elevated expression of alkaline phosphatase (ALP). Phosphate released by ALP is related to suppression of osteoclastogenesis. Alkaline phosphatase can be detected in the clear fluid excreted by gingival crevicular fluid (GCF). ALP is an enzyme that plays a crucial role in bone metabolism and is often used as a biomarker to monitor bone turnover. In the context of orthodontic treatment, understanding ALP activity in GCF can provide valuable insights into the biological processes involved in potential relapse.[6]

Low-level inflammation is the physiopathological basis for coffee use.[7] Various studies have demonstrated that coffee influences cellular and molecular activities, including phosphodiesterase, adenosine receptors, and prostaglandins. Additionally, caffeine can induce osteoclastogenesis through the COX-2/prostaglandin E2 (PGE2) pathway.[8] Consumption of caffeine-containing products may affect bone metabolism.[7] The bone remodeling process is complex, involving both bone resorption and bone formation which requires the coordination of osteoclasts, osteocytes and osteoblasts.[9] During bone formation, an increase in osteoblast activity is accompanied by elevated expression of the enzyme ALP, which is secreted by osteoblasts during the extracellular matrix maturation phase.[10]

As an adjuvant, caffeine can intensify its analgesic and anti-inflammatory effects. Although caffeine acts as a non-selective antagonist of adenosine A1 and A2-receptors, it also functions as a non-selective adenosine-binding agent with adenosine receptors, amplifying its impact. Intracellular signaling events triggered by adenosine A2α receptors subsequently upregulate the COX-2 gene and promote the release of prostaglandin E2 (PGE2). Consequently, caffeine can inhibit COX-2 activity and reduce prostaglandin synthesis.

PGE2 inhibits the formation of RANKL, which subsequently decreases the number of osteoclasts. RANKL is a key molecule that stimulates differentiation and serves as an inducing agent involved in bone destruction. RANKL does not bind to RANK on the surface of osteoclast precursor cells, it triggers a signaling switch called 'tumor necrosis factor receptor-associated factor 6 (TRAF6)'. This inactivation of signaling pathways, including NF-κB and mitogen-activated protein kinase (MAPK), prevents the relapse process from occurring.

Previous studies have shown that alkaline phosphatase (ALP) activity in gingival crevicular fluid (GCF) serves as biological markers during bone remodeling and bone formation in orthodontic tooth movement. ALP activity is higher at tension sites compared to compression sites during orthodontic tooth movement. Relapse can typically be observed at the tension site.[11]

Alkaline Phosphatase is an enzyme that is needed in the bone mineralization process in bone remodeling. Alkaline Phosphatase expression is regulated by signal transduction ERK1/2, Runx-osterix system, and WNT signals. Its role in the bone mineralization process is to prepare an alkaline (basic) atmosphere in the formed osteoid tissue, so that calcium is easily deposited.[12] Alkaline Phosphatase in the oral cavity is obtained from the gingival crevices of the teeth, namely gingival crevicular fluid (GCF).[13] Alkaline phosphatase activity in GCF during orthodontic treatment may be related to treatment time and pressure exerted on periodontal tissues. Alkaline Phosphatase (ALP) levels can be influenced by

several factors, such as age, gender, fluid and chemical drug therapy, pregnancy, smoking.[14]

Previous studies suggest a correlation between coffee consumption and increased Alkaline Phosphatase (ALP) levels following orthodontic retention treatment. The dose of caffeine intake, according to the Food Drug Administration (FDA) recommendation, is 100-200 mg/day, while the Indonesian National Standard (SNI 01-7152-2006) sets maximum caffeine limit at 150 mg/day for food and beverages and 50 mg/day for specific products. Caffeine, a methylxanthine compound, exhibits anti-inflammatory effects by modulating cytokines (IL-4 and IL-10).[15]

The effects of coffee has been widely studied for orthodontic tooth movement; however, only a few has studied the effect of coffee on orthodontic relapse. The purpose of this study is to analyze the effect of coffee administration on relapse following orthodontic retention by evaluating alkaline phosphatase levels.

METHODS

This experimental study used quasi-experimental design and was conducted at the integrated Biomedical Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung. Based on the Federer formula, 36 male *Sprague-dawley* rats weighing 250-300 gram were placed in eight plastic cages and acclimated to a 12-hour light/dark cycle for seven days with free access to water and standard laboratory rat chow. Animal weight and water intake was monitored every other day, and the amount of coffee powder dissolved in 3 ml distilled water and aquadest in each experimental group was determined accordingly. The coffee solution was administered orally via a gastric tube once daily before 9 AM. The controls ingested plain water with no added substance. Water supply was provided every throughout the study. Robusta coffee, characterized by a smooth texture and a more bitter taste, was used in this study.

Orthodontic treatment was administered according to the method described by Shirazi et al. Briefly, each animal was anaesthetized by intraperitoneal injection of 25 mg/kg ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 8 mg/kg xylazine (Rotexmedica, Trittau, Germany).

All animals were confirmed to be in stage 3 (surgical stage) of anesthesia before orthodontic appliance treatment. Edgewise brackets with a slot of 0.022 mm (Marquis™, Ortho Technology®, USA) were applied on the mandibular central incisors, with a distance of 2.1 mm horizontally. A rectangular 0.016 x 0.020-mm wire and a NiTi open coil spring (0.010"×0.030", an initial length of 5.3 mm, compressed length 2.1 mm). were used to deliver a 35 cN orthodontic force, measured using a dental force gauge (Japan). After 14 days of orthodontic tooth movement, the NiTi open coil spring appliances were removed, and the rats were randomly divided into four groups: Day 0, Day 3. Day 7 and Day 14.

The animals had ad libitum access to food and either coffee solution or plain water. Animal weight and water intake were monitored every other day, and accordingly, coffee concentration was adjusted to supply daily doses of 50 mg or 100 mg per rat in the respective experimental groups. Based on previous measurements and consistent with observed intake, each rat consumed approximately 34-36 ml of water per 24 hours. Gingival crevicular fluid samples were collected on days 0, 3, 7, and 14 after removal of the orthodontic appliances (after retention period). Before sampling, the tooth area was cleaned with cotton pellets to remove debris and supragingival plaque. The gingiva was isolated with cotton rolls, and a dental air blower was used to dry the gingival sulcus. Paper points were inserted into the gingival sulcus (orthodontic relapse side) of each incisor to a depth of 1-2 mm for 30 seconds. Each paper point was then transferred to a 1.5 mL Eppendorf tube containing 350 µl of Phosphate-buffered

Saline (PBS). The tubes were centrifuged at 4000 rpm for 10 minutes. Alkaline Phosphatase levels were measured using a UV-Vis Spectrophotometer at a wavelength of 405 nm in the Integrated Biomedical laboratory, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang.

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using a two-way ANOVA followed by Tukey' post hoc test. Statistical significance was observed at $p < 0.05$.

RESULTS

All rats exhibited normal behavior and survived to the end of the experimental period. Mean relapse after orthodontic retention in the three experimental groups is presented in Table 1.

Table 1. The average of ALP level

Groups	Alkaline Phosphatase Levels (ALP) (IU/I) ± SD			
	Day-0	Day-3	Day-7	Day-14
Aquadest	7,363 ± .555	7,396 ± .562	7,296 ± .137	8,500 ± .504
Coffee 50 mg	8,686 ± .245	8,923 ± .130	9,826 ± .465	11,003 ± .475
Coffee 100 mg	8,583 ± .405	8,996 ± .307	11,273 ± .350	12,756 ± .479

Coffee consumption influenced gingival crevicular fluid ALP levels in each experimental group at each time point. Two-way ANOVA revealed a statistically significant effect ($p<0.05$) for each group. This indicates that there were significant differences in the mean ALP levels observed at days 7 and 14 across the different treatment groups.

Table 2. *Post Hoc Tukey test*

Group	Day – 0	Day – 3	Day – 7	Day – 14
Aquades		0,001	0,001	0,001
Coffee 50 mg			0,001	0,001
Coffee 100 mg				0,001

These results are supported by statistical analysis with a p value <0.000 for each experimental group and $p<0.00$ for control group.

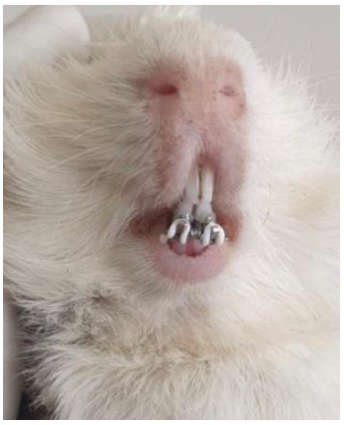

Figure 1. Appliance placement

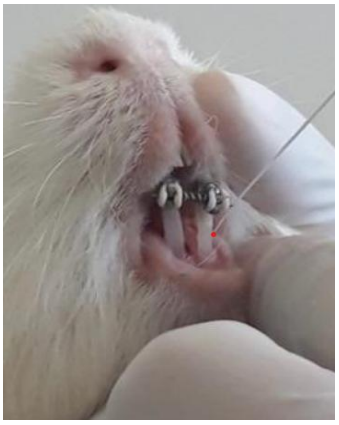

Figure 2. Stabilization stage and gingival crevicular sampling with paper point

Figure 3. Coffee administration to *Sprague-dawley*

Figure 4. Measurement post relapse

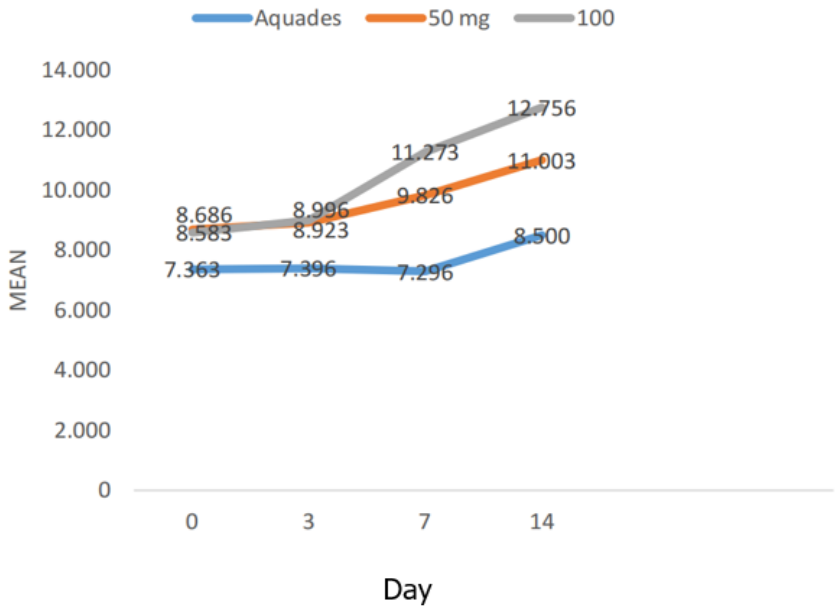

Figure 5. Average of Alkaline phosphatase levels (IU/I)

DISCUSSION

Orthodontic treatment relies on bone turnover, which can be affected by the consumption of various substances and drugs.[4] In this study, we investigated the effect of caffeine on relapse after orthodontic retention and found that caffeine reduced post-orthodontic retention relapse in rat teeth.[6] In this study, the 100 mg caffeine group exhibited the highest ALP levels compared to the 50 mg caffeine and distilled water groups during relapse after orthodontic retention. As shown in Table 1, the highest ALP levels were observed on day 14.

Increased ALP levels are influenced by elevated osteoblasts activity during bone formation, which, according to previous research, occurs following osteoclast resorption and can last from 10 to 21 days. ALP can be obtained from clear fluid excreted by gingival crevicular fluid (GCF).[15] The results of previous studies showed that caffeine has a biphasic dose-response effect on osteogenesis. Low caffeine concentrations exhibit pro-osteogenic effects, while high concentrations exhibit anti-osteogenic effects.[19]

Bone remodeling is a biological process that occurs in response to acute inflammation in periodontal tissue. This process involves coordinated bone resorption and apposition by osteoclasts, osteocytes and osteoblasts.[16] Osteoblasts are bone-forming cells responsible for the secretion and synthesis of bone matrix.

Relapse is influenced by several osteoblast factors and osteoclast-mediated bone resorption around the tooth. Defined as the return of the teeth to their pre-treatment position, relapse is a physiological response of the supporting tissues to the forces applied during orthodontic treatment. In the early phase of relapse, an acute inflammatory response occurs, along with periodontal vasodilation after orthodontic appliance removal.[2]

Osteoblast cells in periodontal tissue form new bone in the new tooth position, contributing to strength and stability needed to maintain the new tooth position. Increased osteoblast activity during bone formation is associated with increased expression of Alkaline Phosphatase (ALP) expression.[17] ALP, a glycoprotein involved in bone mineralization and cementum formation is released during orthodontic treatment and can increase the number of osteoblasts.[18]

Caffeine's potential for inhibiting osteoclastogenesis might be explained through its modulation of NF-kB activity. Specifically, a 10 µg/mL caffeine concentration completely inhibited RANKL- and TNFα-induced through NFkB luciferase activity. TRAF6 plays a critical role in RANKL-induced osteoclastogenesis. Caffeine treatment significantly diminished TRAF6 induced-NF-kB luciferase activity without changing TRAF6's protein levels. These findings suggested that RANKL, TNF-α and TRAF played important roles in activating NF-kB to induce osteoclastogenesis, and that caffeic acid has a strong capacity to inhibit this signal pathway.[16]

The caffeine content in coffee has an antioxidant effect to reduce oxidative stress in osteoblasts.[23] This antioxidant capacity is attributed to the presence of polyphenolic compounds, including chlorogenic acid (CGA), caffeic acid, and ferulic acid. Ferulic acid in caffeine also has anti-inflammatory properties.[24] The stabilization phase or retention phase, a critical period of passive treatment following active orthodontic tooth movement, serves to maintain the achieved tooth position, allowing the tooth supporting tissue to remodel perfectly, thus ensuring long-term stability. Research conducted in animal models have shown osteoblast proliferation during the second day of retention occurring, with peak mineralization occurring on day 4and maturation on day 7. If the stabilization phase is carried out for 2 days, the results of preventing relapse will be less than optimal.[3]

Research indicate that caffeine increases osteoprotegerin (OPG) levels by promoting the osteogenic potential of osteoblasts. For instance, caffein administration (50 mg.kg) to pregnant mice increased OPG levels in their offspring, attributed to enhanced OPG production by osteoblasts. The *In vitro* studies have shown that administering low-dose caffeine (0.1 mM) to primary derived adipose stem cells (ADSCs) and bone marrow stromal cells increases osteoblast differentiation through the markers ALP, Osteocalcin, OPG, and RUNX2.Clinical recommendations from this study in patients who have completed orthodontic treatment in addition to wearing retainers, coffee consumption can be recommended, although further research, including histological analysis of relapse after orthodontic retention, is needed. .

A limitation of this study is the high risk of bracket detachment in rodents. Therefore, specialized brackets designed for rat dentition are necessary for future research.

CONCLUSION

Coffee administration increased alkaline phosphatase (ALP) levels during relapse after orthodontic retention in rats. A 100 mg of c coffee concentration exhibited a pro-osteogenic effect on orthodontic relapse in this model. Further investigation is needed to elucidate the underlying mechanisms. Generalization of the results of animal studies to humans should be performed with caution.

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