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Enhancement of transforming growth factor-beta 1 levels during orthodontic relapse after nanoemulsion carbonated hydroxyapatie - statin administration in rats

Yunita Pransisca[1], Niswati Fathmah Rosyida[2], Sri Suparwitri[2], Ananto Ali Alhasyimi[2]

[1]Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Orthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

Background and objectives. Relapse is acknowledged as a substantial failure subsequent to orthodontic correction. Transforming growth factor-beta 1 (TGF-β1) modulates osteoblastogenesis and stimulates β-catenin signaling. The purpose of this investigation was to assess the impact of nanoemulsion carbonate apatite (CHA) -statin on TGF-β1 levels in rats undergoing relapse.

Materials and methods. A total of forty-eight rats (n = 48) were allocated into four distinct groups: control, CHA, statin, and CHA-statin. To apply 30g of mesial traction for seven days, a closed-coil spring was extended from the first maxillary molar on the right maxilla to the incisor. Seven days passed throughout that CHA hydrogel, statin and CHA-statin nano emulsion were administered every three days. Debonding the appliances subsequently permitted relapse. TGF-β1 levels were measured utilizing an enzyme-linked immunosorbent assay (ELISA) on days 0, 1, 7, and 14 subsequent to debonding. An analysis was conducted on the collected data utilizing ANOVA and a Tukey's test ($p < 0.05$).

Results. On day seven of the relapse phase, the control group had the lowest average; the difference was significant ($p < 0.05$), followed by Group CHA, Group St, and Group CHA-St. Average TGF-β1 levels between the groups did not differ statistically significantly ($p > 0.05$) on days 0 and 1 of orthodontic relapse movement. Out of all the groups, the one that got CHA-statin nanoemulsion had the highest mean values. Significant mean differences ($p < 0.05$) from the highest average of group CHA-St, group CHA, group St, and the control group were found on day 14.

Conclusions. The findings suggested nanoemulsion CHA-statin may raise TGF-β1 levels during orthodontic relapse.

Keywords: orthodontic relapse, carbonate apatite, nanoemulsion, simvastatin, TGF-β1

INTRODUCTION

Orthodontic relapse is the tendency of teeth to shift back to their original points after orthodontic treatment, which is considered an unfavorable consequence [1]. A prior study documented relapse rates following orthodontic treatment to be around 70%–90% [2]. A negative effect of the supporting tissues, relapse is regarded as a serious and challenging orthodontic treatment issue. Finding methods to prevent relapse or improve retention requires us to better understand the relapse process [3]. The cause of relapse movement remains uncertain due to its complexity. An animal study showed that osteoclastogenesis plays a crucial role in relapse [4]. Osteoblastogenesis is thought to oppose osteoclastogenesis [5]. Osteoblasts are the main single-nucleus cells in bone tissue that play a crucial role in forming alveolar bone and maintaining the integrity of a repositioned tooth [6]. Transforming growth factor-beta 1

Corresponding author:
Ananto Ali Alhasyimi
E-mail: anantoali@ugm.ac.id

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(TGF-β1) is essential in the process of osteoblast formation. TGF-β1 has the ability to stimulate cell growth and differentiation of stem cells into osteoblasts [7]. Fluctuations in TGF-β1 levels are crucial for the reorganization of periodontal tissue in orthodontic tooth movement and can significantly enhance bone formation [8].

Orthodontic relapse prevention typically involves the mechanical use of a retainer. Hawley-retainer removable appliances, known for their popularity, are effective in maintaining the desired occlusion. Removable devices necessitate heightened collaboration and reliability from the patient in terms of upkeep and usage; alternatively, a fixed retainer may be more suitable [9]. Fixed retainers are frequently used in orthodontic retention because they require minimal patient involvement, are highly effective, aesthetically pleasing, and suited for long-term retention. Their rigorous bonding requirements and tendency to negatively impact periodontal health limit their utilization [10]. Pharmacologically, orthodontic relapse prevention is also enhanced by bisphosphonates and similar medications. However, bisphosphonates elicit adverse effects in patients, such as osteonecrosis of the mandible bone and oral intolerance [11].

To date, tissue engineering techniques have also been recommended for the purpose of manipulating alveolar bone remodeling, preventing orthodontic relapse, and improving tooth position stability [3]. The utilization of biological stimulants is considered a feasible method for the advancement of regenerative therapy [12]. Carbonate apatite (CHA) is widely regarded as an exceptional biomaterial for enhancing alveolar bone remodeling due to its structural resemblance to the interconnected porous architecture of bone [13]. CHA's capacity to function as a drug delivery system for protein delivery into living cells has also garnered increased interest [14].

Recently, simvastatin, a hypolipidemic agent, has been speculated to potentially function as a therapeutic agent for osteoporosis. Known as statins, these medications represent a novel approach to treating osteoporosis; they prioritize the formation of new bone to replace damaged bone [15,16]. Lee et al. [17] observed that local application of statins to the mandibles of rats resulted in increased bone formation and a greater maximal force to fracture. According to the findings of Han et al.[18], postorthodontic relapse can be reduced with systemic simvastatin administration by inhibiting osteoclasts' bone-resorbing activity and stimulating bone formation. In addition, its remarkable safety profile over an extended period of time makes simvastatin a viable agent for implementation in orthodontic treatment [19]. To achieve statin effects on bone, large dosages are required, which may increase toxicity and adverse effects such as rhabdomyolysis [20]. Simvastatin can be administered locally via hydrogel to circumvent its hepatotoxic characteristics and improve its efficacy for bone repair [21]. The hydrogel is biocompatible, can sustain drug delivery, and is straightforward to manage [22]. Nanoemulsions have grown in popularity as prospective drug delivery systems, either as topical administration systems or as bioavailability enhancers for poorly water soluble active pharmacological components [23]. Currently, the combination of CHA-statin has never been attempted. The purpose of this investigation was to assess whether intrasulcular administration of the nanoemulsion CHA-statin could elevate TGF-β1 levels in rats undergoing experimental orthodontic relapse. This study hypothesizes that TGF-β1 levels are increased during orthodontic relapse in rats by nanoemulsion CHA-statin.

MATERIALS AND METHODS

Experimental animals

The animal experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee of the Faculty of Dentistry, UGM with letter number 41/UN1/KEP/FKG-RSGM/EC/2023. A total of 48 male (n= 48), 8-week-old Sprague-dawley rats, weighing 250±25 g, were enrolled. Animals were randomly divided into four groups as follows: a control group (group C) without any treatment after orthodontic tooth movement (OTM); a CHA hydrogel group (group CHA); a statin nanoemulsion group (group St); and a CHA-statin nanoemulsion group (group CHA-St). Each group was randomly divided into five subgroups (n = 3) according to the observation day.

In every experiment, anesthetized rats were utilized. A week of acclimatization was conducted prior to the commencement of the experiments so that the animals could become accustomed to their new environment and laboratory diet. Every individual rat was housed in a polycarbonate enclosure that followed a standardized light-dark cycle lasting 12 hours and 12 hours, with a temperature of 23.2°C and a relative humidity of 55%±5%. The rats were provided with tap water and a pellet diet (expanded pellets; Stepfield, UK) ad libitum in order to reduce the possibility of bracket detachment and prevent any challenges with feeding while undergoing OTM.

Material preparation

In order to manufacture CHA hydrogel, type-I gelatin (Nitta Co., Japan), sodium citrate, and distilled water were combined. Following the addition of calcium hydroxide, this mixture was agitated for

one hour with a magnetic stirrer. Phosphoric acid was dissolved in 50 millilitres mL of distilled water, then carefully added to the gelatin mixture. The specimen was next ground up and passed through a 32 µm mesh screen. Following synthesis, 50 mg of CHA was added to 5 mL of aquades to form hydrogel CHA. Simvastatin nanoemulsion gel was prepared by combining 100 µl oleic acid, 400 µl Tween80, 25 mg simvastatin, and 2 ml distilled water with a gel base composed of 25 mg carbomer and 2.5 ml hot water in a 1:1 ratio. Simvastatin nanoemulsion gel (0.05mg/10µl) and CHA (0.1mg/10µl) were mixed in a 1:1 ratio to create the CHA-Simvastatin nanoemulsion gel.

Orthodontic tooth movement and relapse movement

The rats were anesthetized by intramuscular injection with a concoction of ketamine (35 mg/kg BW) and xylazine (5 mg/kg BW). Following anesthesia, a closed and short nickel-titanium coil spring (1 mm in diameter, 0.14 mm thickness and 6 mm length; Ormco, USA) was secured using a split-mouth design. One end of the spring was positioned between the right maxillary first molar and the incisor during installation. By creating a perforation between the two incisors using a spherical drill, the opposite end of the spring was affixed to the maxillary incisor in order to accommodate the 0.25mm ligature wire (American orthodontic, USA). An inverted conical drill (HM 1006 ± FG Meissenger-Germany) was utilized to create a distal and mesial groove in the maxillary incisors. These grooves were intended to accommodate a ligature steel wires that were secured with composite resin (Filtek™ Z250 XT-3M St. Paul, MN, USA). The orthodontic appliance was used to move the first molar in the mesial direction, while the second and third molar were ligated and fixed (Figure 1). Dynamometer calibration indicated an initial force of 50 gram force/cN (MedKraft Orthodontics, USA). With flowable composites, the closed-coil spring that served as a retainer was blocked out to make it passive. A stabilization period of seven days was devoted to maintaining a distance of ± 1.5 mm. Following the stabilization period, both the wire and closed-coil spring were eliminated, thereby permitting the molars to initiate relapse movement.

Intrasulcular application of 15 µl of CHA hydrogel (CHA group), nanoemulsion statin (St group), and nanoemulsio CHA-statin (CHA-St group) into the distal side of the gingival sulcus of the incisor was administered every three days to the treatment groups on days 0, 4, and 7 of the stabilization period under general anesthesia.

TGF-β1 levels analysis

After collecting gingival crevicular fluids (GCF) samples, the gingival sulcus of every rat was dehydrated using gentle airflow. Alternatingly throughout the relapse movement, GCF samples were collected from each group on days 0, 1, 7, and 14 utilizing a #20 paper point. The paper point was inserted with care, approximately 1 mm into the gingival sulcus of the first molar on the distal side (pressure area). It was left in place for a duration of 60 seconds. The collection process was duplicated twice. Then, two paper points that had been immersed were transferred into a 1.5 mL Eppendorf tube that contained 350 L of physiological saline solution. The tube was subsequently centrifuged for 5 minutes at 4°C and 2000 rpm.

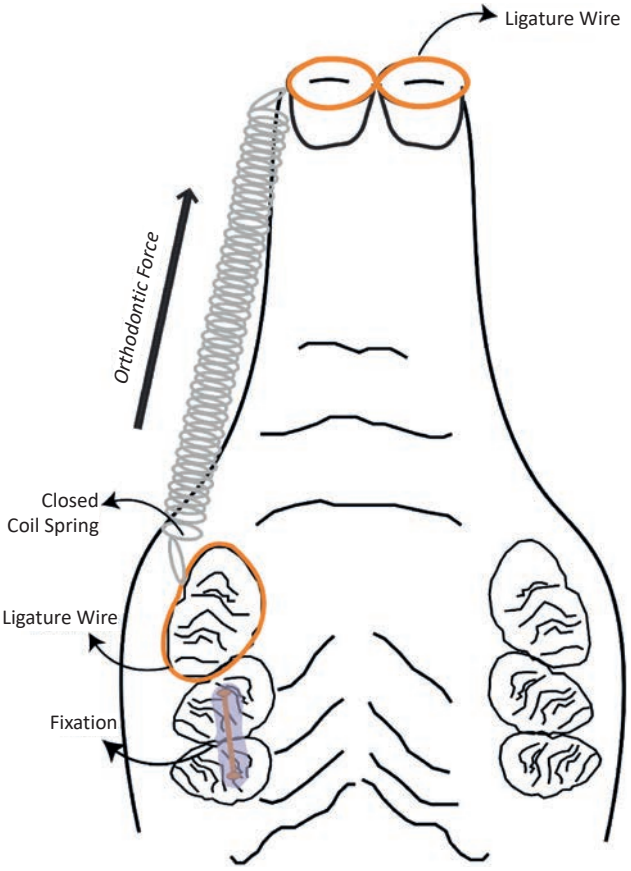

FIGURE 1. Design of an orthodontic tooth movement for the rat model

Using an enzyme-linked immunosorbent assay (ELISA), TGF-1 levels during relapse progression were determined. The quantitative anti-TGF-1 antibody-specific ELISA reagent (RK00055, ABclonal, Düsseldorf, Germany) was utilized for the analysis. A comparison was made between the total quantity of the transcription factor and its standard curve. Using a microplate reader, the optical density of the solutions was ascertained at 450 nm. The cumulative concentration of TGF-β1 was denoted in picograms per milliliter (pg/mL).

Statistical analysis

By employing two-way ANOVA, the data acquired in this research were subjected to statistical analysis

in order to identify distinctions and interactions among groups. Subsequently, significant differences between groups were ascertained using Tukey's honest significant difference (HSD) test. A p-value below 0.05 was deemed to indicate statistical significance. The analyses were conducted utilizing version 21 of the Statistical Package for the Social Sciences (IBM, USA).

RESULTS

The animals showed no signs of distress in all experimental procedures . In addition, administering the material at the prescribed dosage did not cause any overall toxicity, such as edema, and did not impact the animals' body weight. The levels of TGF-β1 exhibited variation among the different groups. Table 1 displays the mean and standard deviations of TGF-β1 levels in GCF that were assessed using ELISA. The Shapiro-Wilk normality test and Levene's homogeneity test confirmed that the data is normally distributed and homogenous ($p > 0.05$). The two-way ANOVA analysis revealed a substantial increase in TGF-β1 levels. The mean from day 1 to day 7 considerably increased ($p < 0.05$) in all groups. The control group had the lowest average, followed by Group CHA, Group St, and Group CHA-St on day 7 during the relapse phase. Nevertheless, the group that received CHA hydrogel and statin nanoemulsion did not exhibit a difference in the increase of TGF-β1 levels during relapse movement on day 7 ($p > 0.05$).

On days 0 and 1 of orthodontic relapse movement, there was no statistically significant difference ($p > 0.05$) in the average TGF-β1 levels between the groups. However, the group that received CHA-statin naoemulsion had the highest mean values of all the groups. A significant decrease in TGF-β1 levels occurred from day 7 to day 14 in all groups. Day 14 denoted a significant mean difference ($p < 0.05$) from the highest average of group CHA-St, group CHA, group St, and the control group.

DISCUSSION

The present study validates our hypothesis that during orthodontic relapse, nanoemulsion CHA-statin may increase TGF-β1 levels. As compared to the control group, the results indicated that CHA hydrogel, nanoemulsion statin, and CHA-statin treatment may raise TGF-β1 levels during orthodontic relapse. TGF-β1 levels have significant effects on the remodeling of periodontal tissue during orthodontic tooth movement and have a strong ability to stimulate bone formation [24]. The mechanism of relapse following orthodontic movement is identical to that of OTM. During the process of relapse, the side of OTM that was previously under stress now experiences higher pressure. This results in an increase in the differentiation of osteoclasts, which leads to the resorption of bone. On the other hand, the side of the bone that was previously under pressure changes to become the side under tension, promoting the development of osteoblasts and initiating new bone growth [6]. Previous research suggests that osteoclasts continue to cause bone resorption on the pressure side during the retention phase. However, minimal osteoclast activity was observed in the stress area. In summary, they conclude that osteoclast activity may be a plausible factor contributing to orthodontic relapse [25]. Generally, TGF-β1 suppresses the process of osteoclastic bone resorption. TGF-β hinders the attraction of cells that develop into osteoclasts and also directly decreases the activity of osteoclasts in resorbing bone tissue [26]. While preventing additional osteoclastogenesis, TGF-β1 expression on the bone surface encouraged osteoblastogenesis and the formation of new bone. Other factors generated by osteoblast/stromal cells under TGF-β1 regulation may mediate a potential involvement of TGF-β1 in the stimulation of osteoclast apoptosis at the bone surface [27] Murakami et al. [28] and Takai et al. [29] observed a significant and dose-dependent increase in the expression of OPG in osteoblastic cell lines and bone marrow-derived stromal cells following treatment with TGF-β1.

Significant differences in TGF-β1 levels across the groups on days 7 and 14 during the relapse phase show that CHA hydrogel and nanoemulsion CHA-statin treatment is effective in raising TGF-β1 levels. Regarding the mechanism, CHA seems to promote bone remodeling by raising the levels of calcium and phosphate in the surrounding tissue, which

TABLE 1. Results of the two-way ANOVA and post hoc Tukey's HSD test comparing the TGF-β1 level among four groups tested

Parameter	C	CHA	St	CHA-St	P-value	Post hoc comparison
TGF-β1 level						
Day 0	36.21± 2.79	37.49 ± 3.12	36.97 ± 4.13	38.51 ± 2.57	0.086	NS
Day 1	37.53 ± 3.13	38.13 ± 3.75	38.01 ± 3.08	40.61 ± 3.09	0.056	NS
Day 7	44.47 ± 3.21	61.81 ± 2.96	62.07 ± 3.24	69.24 ± 2.98	0.000*	C<CHA,St<CHA-St
Day 14	43.51 ± 4.35	61.19 ± 4.01	59.91 ± 3.13	68.07 ± 3.42	0.001*	C,St,CHA<CHA-St

The values are displayed using the mean along with the standard deviation.
Tested by two-way ANOVA and post hoc Tukey's HSD test; C, the control group; CHA, CHA group; St, statin group; CHA-St, CHA-statin group.
* $p < 0.05$, significant difference between groups; NS, not significant

are required during the production of new bone [30]. Released into the local environment, phosphate and calcium promote osteoblastic growth [31]. TGF-β1 is well recognized to control osteoblast differentiation and proliferation. Among the many ways TGF-β1 promotes osteoblast proliferation are its ability to attract matrix-producing osteoblasts, or osteoblast precursors, by chemotactic attraction and to inhibit osteoblast apoptosis [32]. As one of the osteoclast differentiation factors, the receptor activator of nuclear factor kappa-β ligand (RANKL) expression is suppressed by high TGF-β1 levels. Stated differently, bone mass can be increased, and osteoclast production and activation can be limited indirectly by TGF-β1. The passive orthodontic phase is supposed to have this state to prevent relapse movement [33]. The group that received CHA-statin nanoemulsion exhibited the highest average levels of TGF-β1 among all the groups. Previous research confirms that simvastatin has positive effects on bone via enhancing the differentiation of osteoblasts through the regulation of TGF-β1 expression, which is essential for bone formation [34]. After osteoblasts cease secreting bone matrix, 5% of them transition to osteocytes, 30% to lining cells, and 65% to apoptosis. Bone formation is enhanced by preserving osteoblasts from apoptosis. Statins may also enhance bone formation by inhibiting osteoblast apoptosis through the TGFβ/Smad3 signaling pathway [35]. Nanoemulsion CHA-statin formulation exhibits excellent stability against sedimentation and creaming, making it an optimal choice for formulating lipophilic medicines. This formulation enhances both solubility and permeability [36]. Figure 2 provides a summary of how nanoemulsion CHA-statin enhances TGF-β1 levels.

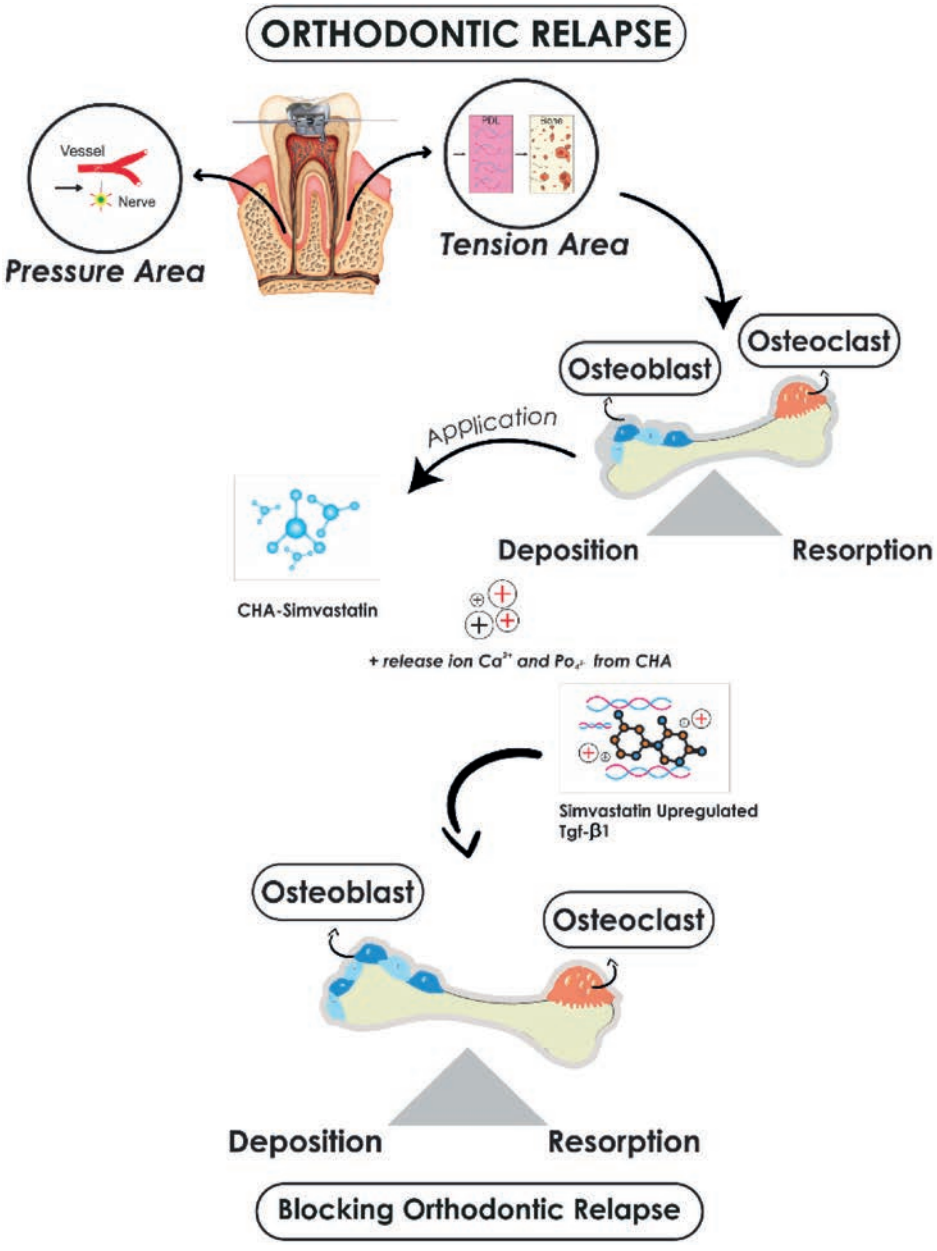

FIGURE 2. Mechanism of nanoemulsion CHA-statin enhances TGF-β1 levels

There was no significant difference in the levels of TGF-β1 between day 0 and day 1 following debonding in all groups. TGF-β1 has biphasic effects on osteoclast maturation, as demonstrated by prior study. TGF-β1 activates nuclear factor kappa B (NF-κB) levels and RANK in osteoclast precursors during the initial phase of the osteoclast maturation process, thereby inducing osteoclastogenesis from hematopoietic precursors. The survival and differentiation of osteoclast precursors into osteoclasts are significantly influenced by the RANKL-RANK interaction. High concentrations of TGF-β1 promote osteoblast OPG levels and decrease RANKL levels at the end stage of osteoclast maturation. OPG inhibits the RANKL-RANK interaction, resulting in the differentiation of osteoclasts into active-inhibited osteoclasts [32]. More intriguingly, preventing TGF-β from suppressing the regained Col-I expression in CD90(+) PDL progenitors delayed PDL collagen recovery and somewhat prevented the early relapse [37]. In the treatment group, the highest TGF-β1 levels were significantly seen on the seventh day after debonding. This is consistent with the study by Alhasyimi et al. [8] that indicates on day 7, osteoblast synthesis and osteoid matrix rich in type I collagen emerge, which also controls osteoid mineralization and, in this setting, causes TGF-β1 levels in osteoblasts to reach their highest peak. TGF-β1 levels decrease on day 14 in all groups. The average TGF-β1 level is expected to decline due to the lag phase. The lag phase is characterized by minimal or absent OTM activity. During this phase, the relapse activities are discontinued for a duration of around 20-30 days. This stage is characterized by the periodontal ligament hyalinization process, which involves removing necrotic tissue by osteoclasts, macrophages, and foreign body giant cells [38].

CONCLUSION

The findings suggested that the use of nano emulsion CHA-statin during orthodontic relapse may lead to an increase in TGF-β1 levels. However, it is necessary to conduct additional and more extensive clinical investigations in order to establish and clarify the effectiveness of nanoemulsion CHA-statin in humans.

Conflict of interest: The authors declare there is no conflict of interests regarding the publication of this paper.

Author's contributions:
Conceptualization, N.F.R and A.A.A; methodology, Y.P., N.F.R., S.S., A.A.A.; software, Y.P.; validation, N.F.R and A.A.A.; formal analysis, Y.P., N.F.R., S.S., A.A.A.; investigation, Y.P., N.F.R., S.S., A.A.A; resources, A.A.A; data curation, Y.P., N.F.R., S.S., A.A.A.; writing–original draft preparation, Y.P., N.F.R., S.S., A.A.A.; writing–review and editing, A.A.A; visualization, Y.P. and A.A.A.; supervision, N.F.R., S.S., A.A.A X.X.; project administration, N.R.R.; funding acquisition, A.A.A.
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