Correlations of Alkaline Phosphatase Expression with Osteoblast Number during Orthodontic Tooth Movement, *in vivo*

Ari Triwardhani[1], Ryan Raditya Tjandra[1], Thalca Hamid[1], Tamara Nitya Ariani[1]

1. Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Abstract

The alveolar bone has capacity adapting to tooth movement. Alveolar bone remodels whilst undergoing orthodontic tooth movement (OTM). There are two mechanisms happened after the administration of orthodontic force, the pressure side undergoes bone resorption, whilst the tension side undergoes bone apposition. Osteoblast activity can be determined from alkaline phosphatase (ALP). The purpose of this research was to investigate the correlations of alkaline phosphatase expression with osteoblast number during orthodontic tooth movement.

The samples consisted of 30 Wistar rats which were selected at random and then split into 5 groups: group 0 (controlled group, no orthodontic tooth movement, day 0), group 1-4 (with orthodontic tooth movement, day 7,14, 21, and 28). The orthodontic tooth movement (OTM) was distal movement, produced using simple coil spring, installed between both upper central incisive with light force. Furthermore, the Alkaline phosphatase expression and osteoblast number was examined in the alveolar bone during OTM in vivo.

Alkaline phosphatase expression was increased significantly 7 days after applying OTM and gradually increasing as the OTM progressed. Osteoblast count was also increased significantly 7 days after applying OTM.

There are correlations of alkaline phosphatase expression with osteoblast number during orthodontic tooth movement. When the number of osteoblasts increases, the ALP expression also increases.

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Introduction

The alveolar bone has capacity adapting to tooth movement. Alveolar bone remodeling is a biological response whilst undergoing orthodontic tooth movement (OTM)[1,2]. This unique mechanism is an uninterrupted and balanced biological mechanism. This mechanism requires highly organized activities of numerous types, one of them is osteoblast[3,4]. There are two mechanisms happened after the administration of orthodontic force, the pressure side undergoes bone resorption, whilst the tension side undergoes bone appoisition[5,6]. Multiple s of force degree, repetition, and period of OTM throughout orthodontic therapy produce considerable impact on the neighboring soft tissue compensation and bone remodeling[7-9].

The initial stage of OTM causes inflammatory reactions, indicated by tissue vasodilatation and migration of white blood s outside the capillaries. This phenomenon established inside gingival crevicular fluid (GCF) of teeth in motion, where inflammatory mediators namely cytokines and prostaglandins significantly increased[10,11]. Synergy among bone formation and resorption throughout OTM produces numerous biochemical or cellular mediators which could be classified as probable biomarkers. Numerous researches have explored possible biomarkers for bone modelling throughout OTM. Bone biomarker namely alkaline phosphatase enzyme (ALP) had generally been related with osseous matter formation. Increased ALP activity had been observed at tension side related with compression side throughout OTM[12-17].

The periodontal ligament is the connective tissue that lies amid root and alveolar bone. It has an important role act as a supporting

Corresponding author:
Dr. Ari Triwardhani,
Department of Orthodontics, Faculty of Dental Medicine,
Universitas Airlanga, Surabaya, Indonesia.
E-mail: ari-t@fkg.unair.ac.id

tissue for teeth and controls the distribution of mechanical stresses[18,19]. Throughout OTM is required balanced repair of the periodontal extracellular matrix close together to support tooth movement to happen, whereas the functional integrity of the periodontium is maintained. During tissue remodeling, extracellular matrix elements can be broken down by proteolytic enzymes or phagocytosis of s in the periodontal ligament[20-22]. Osteoblasts are special s that synthesize bone matrix and manage the coordination of bone mineralization[23,24]. They differentiate from mesenchymal s and work closely with osteoclasts through a continuous cycle that is an essential mechanism throughout OTM[25,26].

Alkaline phosphatase (ALP) is a protein enzyme that hydrolyzed organic phosphatase esters. This enzyme plays important roles in remodeling of periodontal ligament, cementum, and bone homeostasis[12,13]. Alkaline phosphatase (ALP) is attached to membrane inositol-phosphate on the outside of osteoblasts. The action of ALP is an indicator of the existence of osteoblast s and the development of new bone[14,15]. The aim of this research is to investigate the correlations between alkaline phosphatase (ALP) expression and osteoblasts count during OTM.

Materials and methods

The sample consists of 30 healthy Wistar rats age 16-20 weeks weighing 200-250 grams, split into 5 groups (1 control group and 4 treatment groups) and each group consists of 6 Wistar rats (**Table 1**).

No	Group	Days
1	Control	0
2	Treatment 1 (T1)	7
3	Treatment 2 (T2)	14
4	Treatment 3 (T3)	21
5	Treatment 4 (T4)	28

Table 1. Experimental group design in this study.

Orthodontic tooth movement was distal movement, produced using simple coil spring. The coil spring is made using simple wire. Before the insertion, the force is measured using tension gauge to make sure the force produced is 10 gr/mm^2. The coil spring was inserted between maxillary central incisive to deliver distal movement. After 7, 14, 21, and 28 days after coil spring activation, the samples were extracted and prepared using paraffin block. From each paraffin block that were cut, one piece was stained using haematoxylin-eosin, one other piece was used for immunohistochemistry staining. The examination was conducted by means of streptavidin-biotin-peroxidase with label streptavidin biotin (Dako, Carpinteria, USA). Alkaline phosphatase expression was examined quantitatively by means of light microscope at 1000x magnification and then counted using tool image[15,27].

ALP expression and osteoblast number were analyzed statistically using Statistical Package for the Social Sciences Software (SPSS) 20.0 edition (SPSSTM, Chicago, United States). Data distribution was tested using *Kolmogorov Smirnov* test. After that, to determine the differences of ALP expression and osteoblast number on day 0, day 7, day 14, day 21, and day 28, the data was tested using *repeated-measures analysis of variance* (ANOVA) ($p<0.05$). After that, the data analyzed using multiple comparisons, *Tukey HSD* test ($p<0.05$). While correlations test was using *Pearson test* ($p<0.05$).

Results

Microscopic examination showed that there was a difference of alkaline phosphatase expression between the control group and four treatment groups. ALP expression and osteoblast count were calculated quantitatively based on color intensity in each group. The difference was examined from brown colored mass inside cytoplasm (see **figure 1 and 2, Table 2).** The intensity of the brown colored mass is gradually stronger on each group and the strongest showed on the last group, 28 days after being applied of orthodontic force.

The data variant was homogenic from *Levene test* ($p<0.05$) and distributed normally according to *Kolmogorov Smirnov test* ($p>0.05$). The differences between groups are significant according to ANOVA test ($p<0.05$). The control group expressed the lowest mean ± standard deviation alkaline phosphatase (ALP) (2.67 ± 0.816), while group treatment T4 (28 Days) expressed the highest mean ± standard deviation ALP (15.83 ± 2.137). There was a significant increase in ALP expression between control

group and T1 group (7 days), the significant increase also showed between T3 group (21 days) and T4 group (28 days) (**Figure 3).**

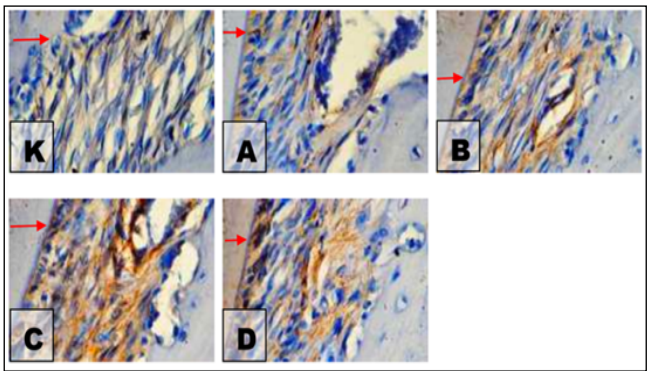

Figure 1. Alkaline phosphatase expression. K. Control group, A. T1 (7 Days), B. T2 (14 Days), C. T3 (21 Days), D. T4 (28 Days). Alkaline phosphatase expression was shown as the brown colored mass on the cytoplasm (red arrow). (Histochemical examination, magnification at 1000x)

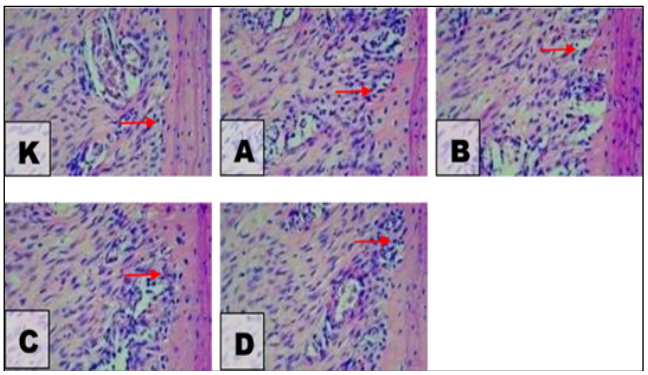

Figure 2. Osteoblast s count. K. Control group, A. T1 (7 Days), B. T2 (14 Days), C. T3 (21 Days), D. T4 (28 Days). Osteoblast was shown as the brown colored mass on the cytoplasm (red arrow). (Histochemical examination at magnification 1000x).

The control group showed the lowest mean ± standard deviation osteoblast count (3.50 ± 0.548), while group treatment T4 (28 Days) showed the highest mean ± standard deviation (17.50 ± 1.049). There was a significant increase in osteoblast number between control group and T1 group (7 days), the significant increase also showed between T3 group (21 days) and T4 group (28 days) (**Figure 4**).

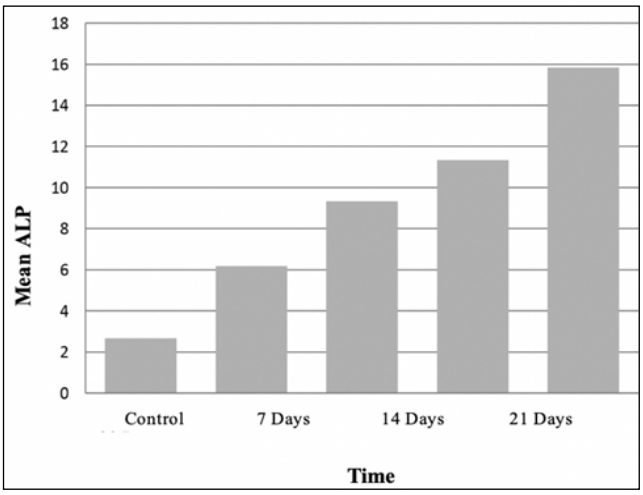

Figure 3. The mean and standard deviation of alkaline phosphatase expression for each groups. The highest of ALP expression was found in 28 Days group, whilst the lowest was found in Control Group.

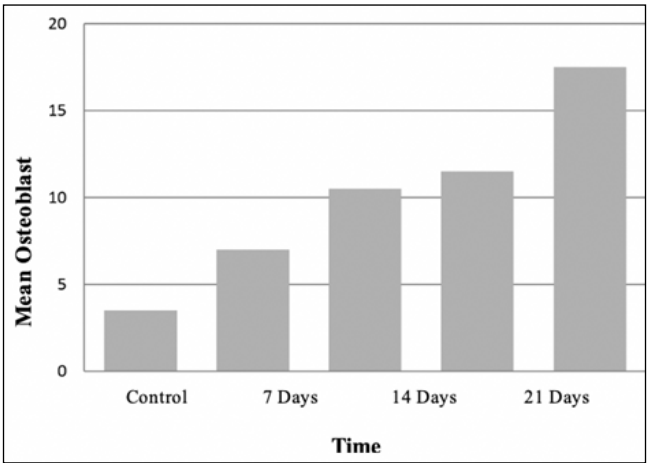

Figure 4. The mean and standard deviation diagram of osteoblast count for each groups. The highest of osteoblast count was found in 28 Days group, whilst the lowest was found in control Group. In addition, the data were examined using *Tukey HSD test* to found the detailed information which groups have significant difference. Every groups on both variables showed significant difference ($p<0.05$), however the difference between group T2 (14 days) and group T3 (21 days) was not significant ($p>0.05$).

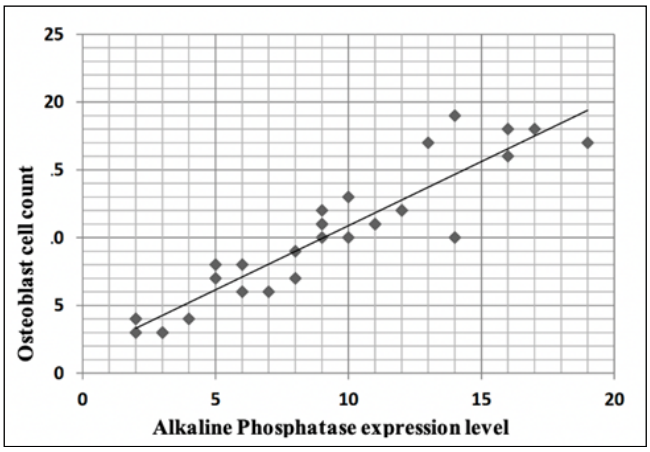

Figure 5. Correlation diagram between alkaline phosphatase expression and osteoblast count. The distribution of points that approach the diagonal line shows a regular pattern.

The correlation between alkaline phosphatase expression and osteoblast count was significant. The coefficient number of correlation is 0.995 which shown that there was a very significant correlation among alkaline phosphatase (ALP) expression and osteoblast count. The result of *Pearson* correlation test of alkaline phosphatase expression with osteoblast count was showed on **table 3** and the diagram on **figure 5** showed the distribution of points that approach the diagonal line shows a regular pattern.

Variable	Group	C	T1 (7 days)	T2 (14 days)	T3 (21 days)	T4 (28 days)
Alkaline phosphatase expression	C					
	T1 (7 days)	0.003*				
	T2 (14 days)	0.000*	0.008*			
	T3 (21 days)	0.000*	0.000*	0.159		
	T4 (28 days)	0.000*	0.000*	0.000*	0.000*	
Osteoblast cells count	C					
	T1 (7 days)	0.000*				
	T2 (14 days)	0.000*	0.000*			
	T3 (21 days)	0.000*	0.000*	0.371		
	T4 (28 days)	0.000*	0.000*	0.000*	0.000*	
*information: significant at p<0.05.						

Table 2. Multiple Comparisons of Tukey HSD test results of alkaline phosphatase expression and osteoblast s count.

	N	Mean ± Standard Deviation
Alkaline phosphatase expression level	5	9.0660 ± 5.00606
Osteoblast cell count	5	10.0000 ± 5.24404
*information: significant at p<0.05.		

Table 3. Pearson's correlation test results of alkaline phosphatase expression with osteoblast count.

Discussion

Previous studies have investigated the biologic marker for bone remodeling during orthodontic tooth movement. During orthodontic treatment, there are many types of force application that caused some tissue reactions, such as bone remodeling. Linkage between bone apposition and resorption throughout OTM produces many types of chemical and cellular mediator that can be identified as potential biomarker[28,29].

There were some studies that analysed biomarker responsible for bone remodelling during OTM, for example alkaline phosphatase (ALP) that linked with bone apposition[12-17].

Based on the different ANOVA test in this study, the value of the significance of ALP expression is 0.000 ($p<0.05$). This value means that the difference in the expression of ALP between each research group is significant. This means that OTM activates the production of ALP. ALP expression indicates biochemistry changes that occurred inside tooth supporting tissue following the implementation of orthodontic force. ALP activities usually higher inside the periodontal tissue than other tissues[15,17].

ALP increased significantly 7 days after applying orthodontic force (Figure 4). Throughout bone deposition period, ALP has been known as biologic indicator of osteoblast activity. After orthodontic force had been applied, there were phases of bone deposition to follow, 1) preliminary phase, lasted for 3-5 days; 2) initial phase, lasted for 5-7 days; 3) late phase, lasted for 7-14 days.[13,14] Previous study also revealed bone formation emerged following resorption of osteoclast that could last from 10 to 21 days[11,28].

Osteoblast count increased significantly 7 days after applying orthodontic force. This result was in accordance with a study, that concluded that development of pre-osteoblasts from mesenchymal had happened more or less 10 hours after inserting orthodontic force. After that, 40-48 hours later, osteoblasts were undergoing differentiation. Osteoblasts reached maximal number after 6 days of OTM. The differentiation and proliferation of osteoblasts could carry on up to 10 days. Osteoblast activity during bone apposition period followed with increased of alkaline phosphatase expression[11,12,15,30].

Conclusions

ALP expression correlates with osteoblast s count because OTM produces ALP that can increased osteoblast proliferation. When the

number of osteoblasts increases, the ALP expression also increases. Further study and analysis are needed with better setting to confirm this study.

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Declaration of Interest

The authors declared zero conflicts of interest regarding this investigation.

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