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Original Article

The effect of hyperbaric oxygen 2.4 absolute atmospheres on transforming growth factor-β and matrix metalloproteinase-8 expression during orthodontic tooth movement *in vivo*

[Efecto de la atmósfera absoluta de oxígeno hiperbárico 2.4 sobre la expresión del factor de crecimiento transformante β y la metaloproteinasa de matriz-8 durante el movimiento dental de ortodoncia *in vivo*]

Arya Brahmanta*, Noengki Prameswari, Budi Handayani, Meralda Rossy Syahdinda

Department of Orthodontic, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia.
*E-mail: arya.brahmanta@hangtuah.ac.id

Abstract

Context: Orthodontic force causes the compression of the periodontal ligament in inhibiting vascularization on the pressure side of orthodontic tooth movement (OTM), while on the opposite side, the periodontal ligament is stretched. Hyperbaric oxygen therapy (HBOT) can increase the dissolved oxygen in the blood, which can affect the tissue regeneration of the periodontal ligament.

Aims: To evaluate the effect of HBO 2.4 absolute atmospheres (ATA) for 3 × 30 minutes with 5 minutes interval for 7 days as an adjuvant therapy to accelerate periodontal ligament remodeling during OTM in *Cavia cobaya*.

Methods: The study was an experimental laboratory research with a completely randomized design. Twenty-four males *C. cobaya* were divided into three groups, (X1) as the control group, (X2) as the group with orthodontic force, and (X3) as the group with orthodontic force followed by HBO 2.4 ATA. Matrix Metalloproteinase (MMP)-8 and Transforming Growth Factor (TGF)-β expressions were measured by using immunohistochemistry. OTM was then examined by applying linear distance and analyzed by employing ratio test ($p<0.05$).

Results: Ratio analysis shows HBO 2.4 ATA for 3 × 30 minutes once a day with 5 minutes intervals for 7 days has increased MMP-8 expressions in the pressure area and TGF-β in the tension area ($p<0.05$).

Conclusions: HBO 2.4 ATA for 3 × 30 minutes once a day for 7 with 5 minutes intervals is able to effectively accelerate the periodontal ligament remodeling process expressed by the increased expression MMP-8, especially within the pressure area and TGF-β in the tension area.

Resumen

Contexto: La fuerza de ortodoncia provoca la compresión del ligamento periodontal al inhibir la vascularización en el lado de presión del movimiento dental de ortodoncia (OTM), mientras que, en el lado opuesto, el ligamento periodontal se estira. La terapia con oxígeno hiperbárico (TOHB) puede aumentar el oxígeno disuelto en la sangre, lo que puede afectar la regeneración tisular del ligamento periodontal.

Objetivos: Evaluar el efecto de HBO 2.4 atmósferas absolutas (ATA) durante 3 × 30 minutos con un intervalo de 5 minutos durante 7 días como terapia adyuvante para acelerar la remodelación del ligamento periodontal durante la OTM en *Cavia cobaya*.

Métodos: El estudio fue una investigación de laboratorio experimental con un diseño completamente al azar. Veinticuatro machos de *C. cobaya* se dividieron en tres grupos, (X1) como el grupo de control, (X2) como el grupo con fuerza de ortodoncia y (X3) como el grupo con fuerza de ortodoncia seguido de HBO 2.4 ATA. Las expresiones de metaloproteinasa de matriz (MMP)-8 y factor de crecimiento transformante (TGF)-β se midieron usando inmunohistoquímica. A continuación, se examinaron los OTM aplicando una distancia lineal y se analizaron empleando una prueba de relación ($p<0,05$).

Resultados: El análisis de relación muestra que HBO 2.4 ATA durante 3 × 30 minutos una vez al día con un intervalo de 5 minutos durante 7 días ha aumentado las expresiones de MMP-8 en el área de presión y TGF-β en el área de tensión ($p<0.05$).

Conclusiones: HBO 2.4 ATA durante 3 × 30 minutos una vez al día durante 7 con intervalo de 5 minutos es capaz de acelerar eficazmente el proceso de remodelación del ligamento periodontal expresado por la expresión creciente de MMP-8 especialmente dentro del área de presión y TGF-β en el área de tensión.

Keywords: orthodontic tooth movement; periodontal ligament remodeling; transforming growth factor-β; matrix metalloproteinase-8; hyperbaric oxygen therapy.

Palabras Clave: factor de crecimiento transformante-β; metaloproteinasa de matriz-8; movimiento dental ortodóntico; oxigenoterapia hiperbárica; remodelación del ligamento periodontal.

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INTRODUCTION

Malocclusion features a high prevalence in Indonesia, which is around 80% among the population. The occlusion is now treated not only as the ratio of contact between teeth but as a dynamic, morphological, and functional relationship between all components of the stomatognathic system, presenting a great influence on chewing, swallowing and speech (Lubis and Laturiuw, 2018; Tita and Median, 2019). Malocclusion can be treated by going through orthodontic treatment (Nugraha et al., 2019). Furthermore, a fixed orthodontic treatment that corrects malocclusion can induce inflammation during orthodontic tooth movement (OTM) (Pramusita et al., 2020).

Meanwhile, orthodontic treatment is a type of treatment performed in the field of dentistry that aims to obtain a pleasing dentofacial appearance aesthetically by removing the crowding, rotational arrangement, and apical deviations from teeth correcting the connections between incisal and creating good occlusion relationships (Proffit and Fields, 2012; Narmada et al., 2019). The problem with orthodontic treatment is the duration of the treatment, which requires a long time, a minimum of 6 months of treatment. Moreover, patient's quality of life and self-esteem can be harmed as a result of fixed appliances use, as they may lead to pain, inflammation, discomfort, and troubles related to their daily routine (Pacheco et al., 2015; Rahmawati et al., 2020). Thus, a number of different techniques have been suggested to accelerate OTM (Tsichlaki et al., 2016).

As one of the efforts that might be able to accelerate OTM is the administration of hyperbaric oxygen therapy (HBOT), which has been known in advance as a treatment in the process of wound healing in tissues. HBOT is a method of treatment by inhaling pure oxygen (100%) continuously on the body with air pressure greater than normal atmospheric pressure (Tsichlaki et al., 2016). Thus, HBOT is a treatment method in medicine that uses high-pressure oxygen. Patients inhale pure oxygen, 100% oxygen at an air pressure of more than 1 absolute atmosphere (ATA) in a high-pressure air chamber (RUBT) or hyperbaric vessel, caisson. The therapeutic function of HBO improves the concentration of oxygen in the blood, the function of cellular respiration and normal tissue, and it is necessary for various biochemical enzymatic reactions (Mathieu, 2006; Neuman and Thom, 2008; Jain, 2009). HBOT enhances the dissolved oxygen in the blood and produces the high partial pressure of oxygen (PO_2). A rise in PO_2 affects the oxygen pressure for tissue regeneration, which will then accelerate collagen and ATP synthesis, and osteoblastic and osteoclastic activity (Okubo et al., 2001).

Any cellular changes in OTM include modifications in cells, cell-matrix, gingival crevicular fluid (GCF), which is a biomarker of the periodontium state during OTM (Brahmanta et al., 2016a; Rahmawati et al., 2020). The remodeling response is mediated first by the periodontal ligament. In order for teeth to move, periodontal ligaments must form osteoclasts that resorb bone adjacent to the periodontal ligaments in the pressure side, whereas the osteoblast tension side formed by progenitor cells produces the new bone (Khrisnan and Davidovitch, 2006; Hernawan et al., 2020). If the pressure is applied long enough to the teeth, there will be the remodeling of periodontal structure, including the alveolar bone. Related to the oxygen administration, vascularization performs an important role in the process of bone remodeling and as well as the oxygen pressure in it, which also contributes a shared role (Brahmanta et al., 2016b). The administration of mechanical force with orthodontic pressure to the periodontal ligament will also cause changes in blood vessels, both diameter and number of blood vessels, and transformations in the endothelium as a signal in tissue remodeling (Nareswari et al., 2019; Nugraha et al., 2020). Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor-β (TGF-β) as the growth factors have been identified to play a major part in collagen synthesis, which is important in tissue remodeling (Khrisnan and Davidovitch, 2006; Nareswari et al., 2019; Inayati et al., 2020).

Moreover, teeth will move after getting an exerting constant pressure for extended periods of time. Orthodontic appliances that are activated can exert pressure in the direction, which are desired by the operator in order to correct the malocclusion. Pressure on the teeth will cause changes in the periodontal tissue and alveolar bone (Hisham et al., 2019; Inayati et al., 2020). Furthermore, OTM is a process in which the application of a force induces bone resorption on the pressure side and bone apposition on the tension side (Hernawan et al., 2020; Sitasari et al., 2020). Thus, there will be alveolar bone resorption, which requires the activity of osteoblasts and osteoclasts within the extracellular matrix (Narmada et al., 2019). To facilitate the mobility of these cells, an enzyme is needed to break down collagen, which is the largest component of the extracellular matrix, called collagenase (matrix metalloproteinase, MMP), for example, MMP-8 (Khrisnan and Davidovitch, 2006; Brahmanta et al., 2016a; 2016b). Consequently, the expression of TGF-β and MMP-8 on OTM given HBOT is still not explained. Based on the facts mentioned above, the influence of HBOT 2.4 ATA for 30 × 3 minutes would be investigated for 7 days conducted by the studies on guinea pigs (*Cavia cobaya*) on TGF-β and MMP-8 expression in fibroblast of tension and pressure side during OTM.

MATERIAL AND METHODS

Study design and ethical aspects

This study was an analytical observation, which included the use of a laboratory experiment and randomized post-test only control group. An authorization for this study protocol from the health research ethics committee, Faculty of Dental Medicine, Universitas Airlangga Surabaya, East Java, Indonesia was obtained with the appointment reference number of 151/KKEPK.FKG/X/2014.

Animal preparation

All experimental procedures involving animals were carried out in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines to ameliorate any sufferance of the animals. The guinea pigs (*Cavia cobaya*) were kept in cages with the size of 60 × 40 × 30 cm consisting of 5 guinea pigs in each group per cage. Animals were then fed with a standard pellet diet and *ad libitum* tap water. They were routinely inspected for food consumption and fecal characteristics.

The remodeling of orthodontic tooth movement on animal model and the administration of hyperbaric oxygen therapy 2.4 ATA as an adjuvant

In this experimental study, 25 male guinea pigs with an average age of (3 ± 4 months) and weight around 300-400 g on average were randomly divided into equal groups. The guinea pigs were randomly assigned to three groups: the control group or group (-) with no HBOT treatment; the positive group (+) with only OTM, and the treatment group (P) with OTM and HBOT 2.4 ATA 3 × 30 minutes a day on days 8-14 and given HBOT for 7 days (Brahmanta et al., 2016a; 2016b).

In the treatment group, after installing the separator to induce OTM for 7 days (Fig 1.), HBO was given (in the chamber) for the next 7 days without removing the separator. After the treatment groups (P2 and P3) were put into a monospace animal chamber, the pressure in the chamber was increased to 2.4 ATA for 3 × 30 minutes. After that, it was stopped, and the pressure was lowered to its original state (1 ATA). On the 7th day after the administration of HBOT, which was the 14th day of the study, the maxillary of guinea pigs, including their teeth, were taken out, then the tissue was fixed with 10% formalin buffer solution. Next, decalcification was carried out to dissolve the calcium of the teeth and jawbone so that the tissue could be cut properly. This process would be carried out for 30 days with EDTA at room temperature, which was replaced every day until the tissue becomes soft (Triwardhani et al., 2021). Softness was tested using a needle. After that, the tissue treatment was done, and a paraffin block preparation was made to dye the preparations on TGF-β and MMP-8 expression in the fibroblast of tension and the pressure side during OTM with immunohistochemistry (Brahmanta et al., 2016a; 2016b/b; Triwardhani et al., 2021).

Figure 1. Installed separator in guinea pig (*Cavia cobaya*) as orthodontic tooth movement in animal model.

Statistical analysis

The results of the normality test on all research variables with Shapiro Wilk obtained ($p>0.05$), which showed that all research variables were normally distributed. Furthermore, in finding out that between these groups of studies had homogeneous variance, Levene's test was applied and obtained ($p>0.05$), which expressed that all research variables were indeed homogeneous variance. Then, in calculating TGF-β and MMP-8 expression in fibroblast of tension and pressure side during OTM, a ratio analysis was performed. Additionally, a statistical package for social science (SPSS) version 20 for windows was employed to analyze the data (IBM corporation, IL, USA).

RESULTS AND DISCUSSION

The data description in the form of mean (Mean) and standard deviation (SD) presents TGF-β/MMP-8 expression ratio (Table 1). TGF-β/MMP-8 expression ratio data in the tension and pressure side in each study group showed significant differences ($p<0.05$). The difference in TGF-β/MMP-8 expression ratio between the tension and pressure sides in each study group expressed a significant difference ($p<0.05$), except in the negative control group. Thus, it can be concluded on TGF-β/MMP-8 expression ratio that TGF-β expression in the tension region is higher compared to in the pressure area, and conversely, MMP-8 expression in the higher-pressure area due to the orthodontic mechanical pressure and HBOT 2.4 ATA for 3 × 30 minutes once a day for 7 days. The positive expression of MMP-8 in the pressure and tension sides during OTM in the fibroblast of alveolar bone is shown in Fig. 2. Meanwhile, the positive expression of TGF-β in the pressure and tension sides during OTM in the fibroblast of alveolar bone is also presented in Fig. 3.

Table 1. TGF-β/MMP-8 expression ratios in tension and pressure areas in each study group.

Group	Ratio TGF-β/MMP-8				p-value
	Tension area		Pressure area		
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	
Normal	2.65 ± 0.88[b]	1.60-4.50	1.85 ± 0.59[b]	1.25-3.00	0.085
Orto	2.00 ± 0.60[ab]	1.40-3.0	0.60 ± 0.14[a]	0.43-0.80	0.000*
Orto + HBO	1.79 ± 0.37[a]	1.17-2.43	0.79 ± 0.16[a]	0.47-0.93	0.000*
p-value	0.039*		0.000*		

* significant at $\alpha = 0.05$ ($n = 8$).

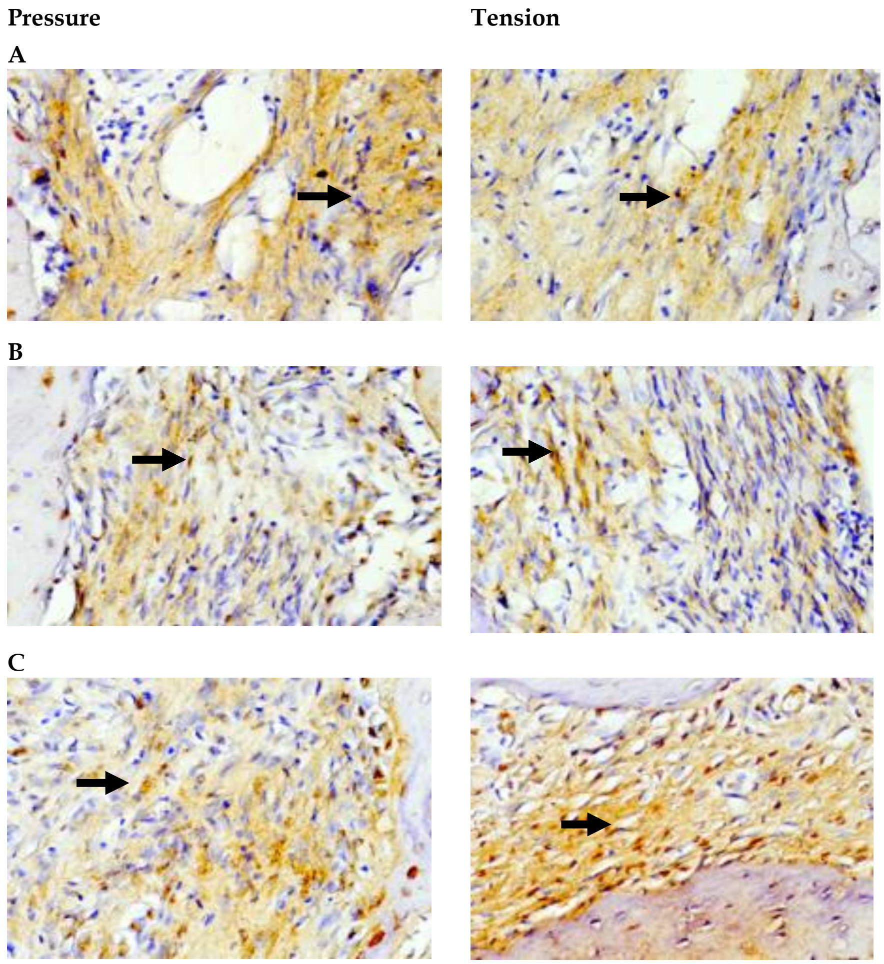

Figure 2. Immunohistochemical features of MMP-8 positive expression (black arrow) in fibroblast cells of *C. cobaya* periodontal ligament tissue in the tension and pressure sides.

(A) Negative control group (no OTM and no HBOT); **(B)** Positive control group (OTM only) and **(C)** Treatment group (OTM and HBOT). (400× magnification).

In the tooth movement caused by an orthodontic mechanical pressure, the pressure of a rubber separator is put with a strength of 0.29 g/cm^2 on day 1 to day 2 and then replaced with a strength of 0.48 g/cm^2. Both are used to move the teeth. OTM results in changes in periodontal tissue, alveolar bone, cementum, the gingival and dental pulp (Sutomo et al., 2012). This movement is characterized by changes in the remodeling of the periodontal ligament tissue (PDL), alveolar bone, pulp and gingiva. OTM alter PDL vascularization, leading to a local synthesis of important molecules such as neurotransmitters, cytokines, growth factors, colony stimulating factors, and arachidonic acid metabolites (Niklas et al., 2013). Related to this, this research on the effects of 14-day OTM was supplemented with the administration of HBOT 2.4 ATA for 3 × 30 minutes once a day for 7 days (on days 8-14.

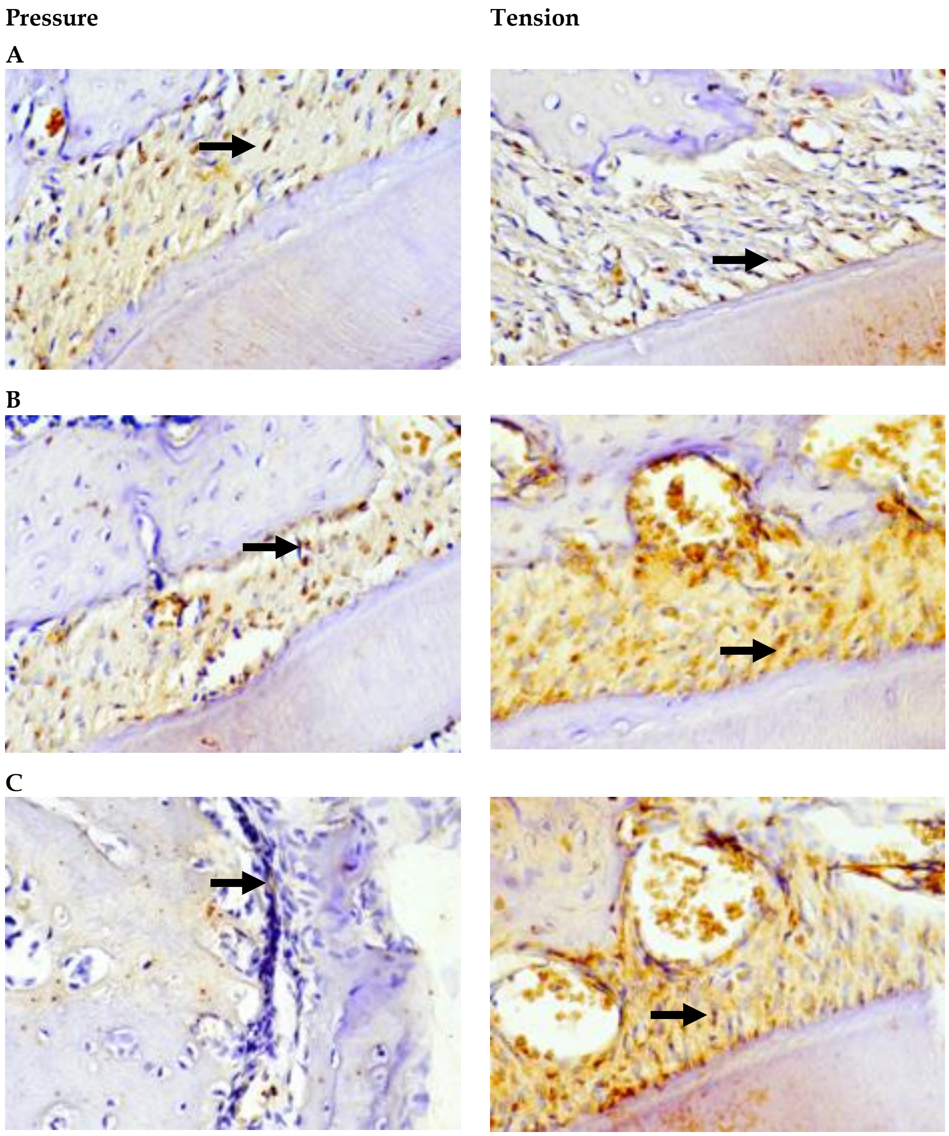

Figure 3. Immunohistochemical features of TGF-β positive expression (black arrow) in fibroblast cells *C. cobaya* periodontal ligament tissue.

(A) Negative control group (no OTM and no HBOT); **(B)** Positive control group (OTM only) and **(C)** Treatment group (OTM and HBOT). (400× magnification).

Furthermore, HBOT will modulate nitric oxide (NO), which plays an important contribution in maintaining blood vessel tone and increasing VEGF, bFGF, TGF-β expression. Then, VEGF, bFGF, TGF-β, together with fibroblasts, will stimulate the synthesis of angiogenesis, which is one of the stages in wound healing (Nareswari et al., 2019; Inayati et al., 2020; Triwardhani et al., 2021). The above effect is related to one of the main benefits of HBOT, which is for wound healing. Blood vessels themselves contribute a significant role in providing oxygen and nutrients, and other materials that are important for bone synthesis as well as sources of osteoblast cells (Domenico et al., 2012, Sitasari et al., 2020). In addition, angiogenesis involves the formation of new branches in blood vessels from pre-existing blood vessels in stages with the onset of vasodilation in response to NO and the increased permeability by VEGF (Mitchell, 2013; Kumar et al., 2013; Inayati et al., 2020; Triwardhani et al., 2021).

CONCLUSIONS

The results of this study analysis prove that the administration of HBOT 2.4 ATA for 30 × 3 minutes once a day as an additional therapy for 7 days can increase the TGF-β expression ratio more in the tension side and conversely increase the MMP-8 expression ratio better in the pressure side during OTM in the *C. cobaya* as documented immunohistochemically.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Brahmanta A	Prameswari N	Handayani B	Syahdinda MR
Concepts or ideas	x			
Design	x			
Definition of intellectual content		x		
Literature search	x			x
Experimental studies	x	x	x	
Data acquisition				x
Data analysis	x	x	x	x
Statistical analysis		x	x	
Manuscript preparation	x			x
Manuscript editing	x			x
Manuscript review	x	x	x	x

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