

THE EFFECTIVENESS OF VITAMIN D PROVISION ON THE EXPRESSION OF FIBROBLAST GROWTH FACTOR-2 UNDER THE ORTHODONTIC MECHANICAL STRESS IN PREGNANT WISTAR RAT (*RATTUS NORVEGICUS*)

Ari Triwardhani, Ida Bagus Narmada*, Faradiah Hayati, Irwadi Djaharuddin, Alexander Patera Nugraha and Dwi Rahmawati

Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.

*e-mail : ida-b-n@fkg.unair.ac.id

(Received 2 May 2020, Revised 11 August 2020, Accepted 26 August 2020)

ABSTRACT : Female patients have the possibility of pregnancy in the course of fixed orthodontic treatment. Pregnant women are often advised by physician to consume various kinds of nutritional support vitamins for the mother and fetus contained, one of which is vitamin D. The outcome of this research is to examine the effectiveness of vitamin D provision on the fibroblast growth factor-2 (FGF-2) expression during orthodontics mechanical stress (OMS) in pregnant wistar rats (*Rattus Novergicus*). Healthy pregnant Wistar rats (*R. novergicus*) 16-20 weeks-old, 200-250 grams body weight then grouped randomly into 4 groups; control group: pregnant rats administered vitamin D without OTM (K7 and K14) and treatment group: pregnant rats with OTM and vitamin D administration (P7 and P14). Nickel Titanium (NiTi) closed coil spring was installed among upper central incisor to the upper first molar to induce OMS with 10g/mm² force. After 7 days or 14 days all animals were sacrificed for pre-maxilla extraction. Immunohistochemically examination was carried out to examine FGF-2 expression in the tension and compressed side. The lowest expression of FGF-2 was found in the P7 group in the tension side (4.7±2.39) and compression side (4.31±1.7). There was no significant difference between groups in the FGF-2 expression on the tension side (p=0.28; p>0.05) and compression side (p=0.14; p>0.05). The vitamin D provision during OMS in pregnant wistar rats (*R. novergicus*) had no significant effect on the expression of FGF-2.

Key words : Fibroblast growth factor, maternal health, medicine, orthodontic tooth movement, vitamin D.

INTRODUCTION

Female patients have the possibility of pregnancy during in the course of orthodontic treatment. Pregnancy is a physiological state that brings various hormonal changes in women. Pregnancy affects not only general health, but also oral health (Narmada *et al*, 2019). During orthodontic treatment with orthodontic mechanical stress (OMS), there are changes in the periodontal ligaments and alveolar bone cellular and biochemical activities, which allows movement of tooth, one of which is fibroblasts in the periodontal ligament (Nugraha *et al*, 2020; Sitasari *et al*, 2020; Rahmawati *et al*, 2020).

There are some vitamin supplements that physicians recommend to consume as a nutritional support for pregnant women, one of which is vitamin D. Vitamin D provision during orthodontic tooth movement (OTM) in the pregnant experimental animal models increases the osteoclast number and receptor activator of nuclear factor κB ligand (RANKL) expression (Narmada *et al*,

2019). However, administration of vitamin D did not have a significant effect on vascular endothelial growth factor (VEGF) expression, angiogenesis, osteoblast number and bone alkaline phosphatase (BALP) expression during the OTM of pregnant wistar rats (Nareswari *et al*, 2019; Hisham *et al*, 2019). FGF-2 is one of kind growth factors that play an important role during wound healing and remodeling of alveolar bone due to injuries (Inayati *et al*, 2020). Thus, the purpose of this research is to examine the effectiveness of vitamin D provision on the FGF-2 expression during orthodontic mechanical stress (OMS) in pregnant wistar rats (*Rattus novergicus*).

MATERIALS AND METHODS

This study obtained ethical qualifications from the ethical clearance committee, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia with number: 733/HRECC.FODM/XI/2019. Female healthy Wistar Rats (*R. novergicus*) 16-20 weeks-old, 200 grams-250 grams of body weight were randomly grouped into 4

groups; control group: pregnant rats administered vitamin D without OTM (K7 and K14) and treatment group: pregnant rats with OTM and vitamin D administration (P7 and P14).

Pregnancy is assisted with the injection of the hormone Pregnant Mare Serum Gonadotropin (PMSG) and Human Chorionic Gonadotropin (HCG) (Sigma Aldrich, US). Female rats have been synchronized to their mating cycle, then collected with one male each, to achieve the same pregnancy period, with treatment less than 21 days after being declared pregnant (Hisham *et al*, 2019). Nickel Titanium (NiTi) closed coil spring (American Orthodontics Corporation, US) was installed among upper central incisor to the upper first molar to induce OMS with 10g / mm² force. Vitamin D was given at the dose of 0.2 mg / kg every 3 days after appliance insertion to the experimental groups. After 7 days or 14 days all animals were sacrificed through cervical dislocation for pre-maxilla extraction. Decalcification using EDTA (OneMed, Indonesia) on extracted pre-maxilla. Immunohistochemically examination was carried out to examine the expression of FGF-2 (Monoclonal antibodies anti-FGF-2, sc-365106, St. Cruz, US) in the tension side and compression side. The observation of FGF-2 expression was done semi-quantitatively with a 400x magnification by means of light microscope (Nikon H600L microscope; DS Fi2 camera 300 megapixels). Positive expression of FGF-2 is characterized by chromogen brown color (Inayati *et al*, 2020). Descriptive statistics are given as Mean \pm Standard Deviation (SD). One-way Analysis of Variance (ANOVA) was done to compare FGF-2 expressions between groups.

RESULTS

The FGF-2 expression with brown chromogen was detected from each group in the tension side (Fig. 1) and compression side (Fig. 2). Expression of FGF-2 data in each group was normally distributed and homogeneous ($p > 0.05$). The greatest of FGF-2 expression was detected in K7 group, while the lowest was in the P7 group in the tension side and compression side (Table 1). The expression of FGF-2 in the compressed side ($p = 0.14$; $p > 0.05$) and tension side ($p = 0.28$; $p > 0.05$) were insignificantly different.

Table 1 : Mean \pm SD of FGF-2 expression in the compression and tension side of each group.

Group	Compression Side Mean \pm SD	Tension Side Mean \pm SD
K7	6.2286 \pm 1.59239	7.4429 \pm 2.35220
K14	7.9000 \pm 3.63272	7.2429 \pm 3.62485
T7	4.3143 \pm 1.70434	4.7000 \pm 2.38817
T14	6.5143 \pm 3.76627	7.2286 \pm 3.25049

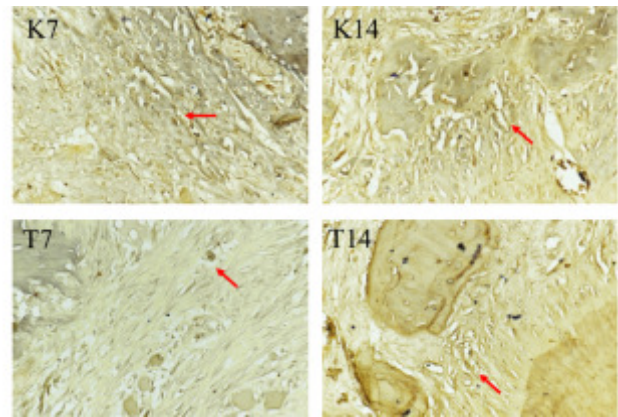


Fig. 1 : Positive expression of FGF-2 in the tension side (red arrow) of each group with 400x magnification.

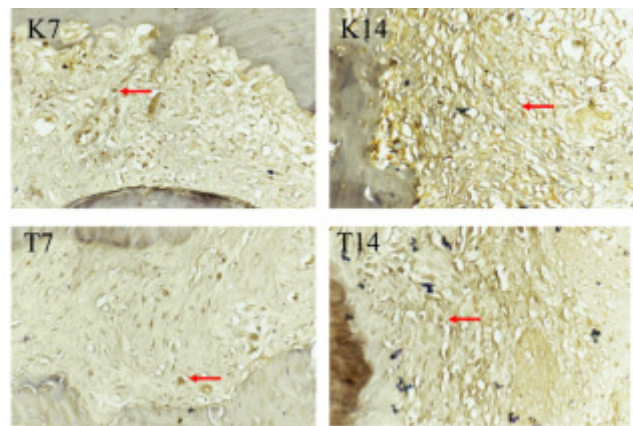


Fig. 2 : Positive expression of FGF-2 in the compression side (red arrow) of each group with 400x magnification.

DISCUSSION

In this study, the greatest expression of FGF-2 was detected in the K7 group, while the lowest was in the P7 group compression side and tension side. There was no significant difference in the expression of FGF-2 in the compression side and tension side between groups. During embryological development, FGF-2 has a role in regulating cell proliferation. FGF-2 is a homeostatic factor and functions to improve tissue and injury response (Inayati *et al*, 2020).

Vitamin D and calcitonin play a role in regulating the amount of calcium and phosphate. Vitamin D is able to stimulate the formation of osteoblasts. Vitamin D in the bone may activate and regulate the transcription of a number of RANKL target genes in osteoblasts that will bind to the receptor activator of nuclear factor κ B (RANK) for osteoclastogenesis and osteoclast activation that crucial for orthodontic tooth movement (Pramusita *et al*, 2020). Excessive vitamin D levels will cause osteoclasts to be more active than osteoblasts so that bone resorption will increase and bone formation will be disrupted (Okazaki *et al*, 2003). Provision of vitamin D in pregnant mice with induction of OMS can increase

RANKL expression and osteoclast numbers, but did not have a significant effect on osteoblast numbers and BALP expression (Narmada *et al*, 2019; Hisham *et al*, 2019). FGF-2 has the effect of inhibiting calcification through a decrease in serum calcium and phosphate. In other words, FGF-2 can reduce calcification by inhibiting the activation of vitamin D (Herlina *et al*, 2017). In a state of hypercalcemia due to an overdose of vitamin D, namely high levels of calcium in the blood due to increased calcium from the digestive tract can lead to calcium excretion in the urine to increase so that the process of bone calcification decreases. This is supported by previous research on hypervitaminosis D which states that the administration of high doses of vitamin D to the embryo causes delayed formation of bone trabeculae. Hypervitaminosis D in pregnant mice can cause a decrease in bone mass and minerals in mouse embryos (Immanuel *et al*, 2017).

The OMS exerted during OTM causes pressure and tensile in the periodontal ligament fibers and cells in the periodontal ligament region in the area of compression and tension. This complex process begins the recruitment of osteoclast and osteoblast progenitors, such as the start of extravasation and the chemoattractant of inflammatory cells (Hernawan *et al*, 2020; Nugraha *et al*, 2019; Rahmawati *et al*, 2020). The initial phase of orthodontic tooth movement always involves an acute inflammatory response characterized by capillary vasodilation and leukocyte migration to capillaries. These migrated cells produce various cytokines. These cytokines stimulate the synthesis and secretion of various substances for target cells such as prostaglandins, growth factors and various cytokines (Puspitaningrum *et al*, 2020; Pramusita *et al*, 2020; Krishnan and Davidovitch, 2006). Acute inflammation that occurs is an initial phase and is exudative. One to two days later the acute inflammatory phase becomes chronic proliferative inflammation involving fibroblasts, endothelial cells, osteoblasts and alveolar bone cells (Meikle, 2006). During this period leukocytes continue to migrate to the periodontal tissue and regulate the process of alveolar bone remodeling. During OMS, cytokines are secreted by mononuclear cells such as Interleukin-1 (IL-1). IL-1 can increase the synthesis and secretion of several substances including prostaglandins or growth factor such as FGF-2 (Henneman *et al*, 2008; Inayati *et al*, 2020).

CONCLUSION

The effectiveness of vitamin D provision during orthodontic mechanical force in pregnant wistar rats (*R. norvegicus*) did not have a significant effect on FGF-2 expression in the tension and compression side.

REFERENCES

- Henneman S, Von den Hoff J W and Maltha J C (2008) Mechanobiology of tooth movement. *Eur. J. Orthod.* **30**(3), 299-306.
- Herlina I, Purwanto B and Sugiarto (2017) The administration of 1,25 Dihydrovitamin D (Calcitriol) effect to fibroblast growth Factor-2 level in patient with kidney disease. *J. Biomedika* **9**(1), 1-12.
- Hermawan R W, Narmada I B, Djaharu'ddin I, Nugraha A P and Rahmawati D (2020) The influence of epigallocatechin gallate on the nuclear factor associated T Cell-1 and Sclerostin expression in Wistar rats (*Rattus norvegicus*) during the Orthodontic Tooth Movement. *Research J. Pharm. Tech.* **13**(4), 1730-1734.
- Hisham P B B M, Narmada I B, Alida A, Rahmawati D, Nugraha A P and Putranti N A R (2019) Effects of Vitamin D in Alveolar Bone Remodeling on Osteoblast Numbers and Bone Alkaline Phosphatase Expression in Pregnant Rats during Orthodontic Tooth Movement. *J. Orofac. Sci.* **11**, 79-83.
- Immanuel T M, Herdiman H and Wargasetia T L (2017) High dose of vitamin D3 supplementation decrease calcification of the femur bone in fetal mice. *Jurnal Kedokteran Brawijaya* **29**(3), 185-189.
- Inayati F, Narmada I B, Ardani I G A W, Nugraha A P and Rahmawati D (2020) Post Oral Administration of Epigallocatechin Gallate from Camelia sinensis Extract Enhances Vascular Endothelial Growth Factor and Fibroblast Growth Factor Expression during Orthodontic Tooth Movement in Wistar Rats. *JKIMSU* **9**(1), 58-65.
- Krishnan V and Davidovitch Z (2006) Cellular, molecular, and tissue-level reactions to orthodontic force. *Am. J. Orthod. Dentofacial Orthop.* **129**(4), 1-2.
- Meikle M C (2006) The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. *Eur. J. Orthod.* **28**(3), 221-240.
- Nareswari R A A R, Narmada I B, Djaharu'ddin I, Rahmawati D, Putranti N A R and Nugraha A P (2019) Effect of vitamin D administration on vascular endothelial growth factor expression and angiogenesis number in orthodontic tooth movement of pregnant Wistar rats. *J. Postgrad. Med. Inst.* **33**(3), 182-188.
- Narmada I B, Husodo K R D, Ardani I G A W, Rahmawati D, Nugraha A P and Iskandar R P D (2019) Effect of Vitamin D during Orthodontic Tooth Movement on Receptor Activator of Nuclear Factor Kappa-B Ligand Expression and Osteoclast Number in Pregnant Wistar Rat (*Rattus norvegicus*). *JKIMSU* **8**(1), 38-42.
- Nugraha A P, Narmada I B, Sitasari P I, Inayati F, Wira R and Triwardhani A (2020) High mobility group box 1 and heat shock protein-70 expression post (-)-epigallocatechin-3-gallate in East Java green tea methanolic extract administration during orthodontic tooth movement in wistar rats. *Pesqui Bras Odontopediatria Clín. Integr.* **20**(e5347), 1-10.
- Nugraha A P, Rezkita F, Putra K G, Narmada I B, Ernawati D S and Rantam F A (2019) Triad Tissue Engineering: Gingival Mesenchymal Stem Cells, Platelet Rich Fibrin and Hydroxyapatite Scaffold to ameliorate Relapse Post Orthodontic Treatment. *Biochem. Cell. Arch.* **19**(2), 3689-3693.
- Okazaki K, Jingushi S, Ikenoue T, Urabe K, Sakai H and Iwamoto Y (2003) Expression of parathyroid hormone-related peptide and insulin-like growth factor I during rat fracture healing. *J Orthop Res.* **21**(3), 511-520.

- Pramusita A, Nugraha A P, Yuliyasari N, Ardani I G A W and Triwardhani A (2020) The Potential Capability of Melatonin to Anticipate Postorthodontic Treatment Relapse: A Literature Review. *Biochem. Cell. Arch.* **20**(Supplement 1), 3061-3066.
- Puspitaningrum M S, Rahmadahani D, Rizqianti Y, Ridwan R D, Ansori A N M, Fadholly A, Susilo R J K, Narmada I B, Ramadhani N F and Nugraha A P (2020) Freeze-Dried Epigallocatechin-3-Gallate And Stem-Cells from Human Exfoliated Deciduous-Teeth Scaffold as The Biocompatible Anti-Relapse Material Post-Orthodontic Treatment: A Review. *Biochem. Cell. Arch.* **20**(Supplement 1), 2935-2942.
- Rahmawati D, Nugraha A P, Ardani I G A W, Triwardhani A and Narmada I B (2020) Role of Hematopoetic Stem Cell In Inflammatory Response during Orthodontic Tooth Movement: A Narrative Review. *Biochem. Cell. Arch.* **20**(Supplement 1), 2879-2882.
- Sitasari PI, Narmada I B, Hamid T, Triwardhani A, Nugraha A P and Rahmawati D (2020) East Java green tea methanolic extract can enhance RUNX2 and Osterix expression during orthodontic tooth movement *in vivo*. *J. Pharm. Pharmacogn. Res.* **8**(4), 290–298.