Original

http://dx.doi.org/10.1590/1981-86372025003120250081

Revista Gaúcha de Odontologia

Effect of simvastatin on Runx-2, Osterix and proinflammatory cytokines gene expression in experimental periodontal disease

Efeito da sinvastatina na expressão gênica de Runx-2, Osterix e citocinas pró-inflamatórias em um modelo experimental de doença periodontal

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ABSTRACT

Objective: This study focused on evaluating the effect of simvastatin on Runx-2, Osterix, interleukins 17, 22 and 23 gene expression in ligature-induced periodontitis

How to cite this article

Saba AK, Teixeira LV, Saba-Chufji E, Mouchrek Júnior JCE, Martinez EF. Effect of simvastatin on Runx-2, Osterix and proinflammatory cytokines gene expression in experimental periodontal disease. RGO, Rev Gaúch Odontol. 2025;73:e20250031. http://dx.doi.org/10.1590/1981-86372025003120250081

artigo alinhado ao objetivo Boa saúde e Bem-estar dos Objetivos de Desenvolvimento Sustentável (ODS)

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in an animal model. **Methods**: Wistar rats (n=20) were randomly assigned to two groups: the simvastatin test group (STG, n=10, daily oral administration of 25 mg/kg simvastatin), and the vehicle control group (VCG, n=10, vehicle,10 ml/kg of saline solution, orally). Periodontitis was induced by ligation around the lower molar (STG and VCG). The non-ligature contralateral molars were used as controls (n=10 from each group). After 14 days, the animals were euthanized, and the bone and gingival tissues surrounding the first lower molar were collected. Runx-2, Osterix in bone tissue, and IL-17, IL-22 and IL-23 in the gingival tissue, were evaluated by real-time polymerase chain reaction. The data were analyzed by Mann-Whitney test (α=5%). **Results**: The Runx-2 expression was similar between the STG and VCG groups (p>0.05), whereas the Osterix expression. was higher for STG (p<0.05). The presence of simvastatin reduced the expression. of IL-17, IL-22 and IL-23 in STG compared with the levels identified in VCG for the same cytokines (p=0.029). **Conclusion**: Simvastatin positively modulates Osterix gene expression and reduces the levels of mRNA of proinflammatory interleukins, which may contribute to avoid bone loss and inflammation promoted by periodontal disease.

Indexing terms: Bone resorption. Cytokines. Periodontitis. Simvastatin.

RESUMO

Objetivo: Este estudo avaliou o efeito da sinvastatina na expressão dos genes Runx-2, Osterix e das interleucinas 17, 22 e 23, em um modelo animal de periodontite induzida por ligadura. **Métodos**: Ratos Wistar (n=20) foram distribuídos aleatoriamente em dois grupos: grupo teste com sinvastatina (STG, n=10, administração oral diária de 25 mg/kg de sinvastatina) e grupo controle com veículo (VCG, n=10, 10 ml/kg de solução salina, via oral). A periodontite foi induzida pela colocação de uma ligadura ao redor do molar inferior (STG e VCG). Os molares contralaterais sem ligadura foram utilizados como controles (n=10 de cada grupo). Após 14 dias, os animais foram eutanasiados, e os tecidos ósseo e gengival ao redor do primeiro molar inferior foram coletados. Runx-2 e Osterix no tecido ósseo, e IL-17, IL-22 e IL-23 no tecido gengival, foram avaliados por reação em cadeia da polimerase em tempo real. Os dados foram analisados pelo teste de Mann-Whitney (α=5%). **Resultados**: A expressão de Runx-2 foi semelhante entre os grupos STG e VCG (p>0,05), enquanto a expressão de Osterix foi maior para o STG (p<0,05). A presença de sinvastatina reduziu a expressão de IL-17, IL-22 e IL-23 no STG em comparação com os níveis identificados no VCG para as mesmas citocinas (p=0,029). **Conclusão**: A sinvastatina modula positivamente a expressão do gene Osterix e reduz os níveis de mRNA de interleucinas pró-inflamatórias, o que pode contribuir para evitar a perda óssea e a inflamação promovidas pela doença periodontal.

Termos de indexação: Reabsorção óssea. Citocinas. Periodontite. Sinvastatina.

INTRODUCTION

Chronic periodontitis consists of an immunoinflammatory response to underlying microrganisms in periodontal tissue, which destroy the alveolar bone, the Periodontal Ligament (PL) and root cementum [1,2]. Bacteria cause bone destruction by releasing products that demineralize bone and incite host cells. These cells respond by secreting inflammatory regulators triggering the host's immunological system and influencing disease progression positively [1,2]. In addition, some of the mediators that act on the immunological system also act on regulating the bone system [1,3].

Besides local environment, the periodontitis progression can be affected by patient's systemic health conditions and drug intake [4]. In this context, statins have emerged as a potential immunomodulatory agent that can modify the anti-inflammatory response [5].

Statins are medicinal drugs that have been largely used in the last two decades to reduce high blood level cholesterol, using 3-hidroxi-3-metilglutaril coenzyme A (HMG-CoA) reductase inhibition. This enzyme triggers mevalonate formation, an important intermediary in cholesterol metabolism [6]. In this respect, statins have been largely employed in cholesterol control [6-10]. Besides its effect as cholesterol-lowering drug [11], many pleiotropic effects have been assigned to statins, including anti-inflammatory (7,12] and immunomodulatory functions [9,11-13], angiogenesis induction [9,11-13].

Moreover, statins are selectively bone-bound, and have come to represent a source of beneficial effects in osteoporosis and fracture treatments [14], considering the action of statins in improving bone density [11] and osteoblasts differentiation [9,11,12,14]

The hypercholesterolemia is a highly prevalence disease in global population, and the statins intake are widely used for its control, especially in patients up 40 years old age in which the periodontal disease is commonly detected. Considering that the statins have anti-inflammatory effects besides to cholesterol lowering, and as the periodontitis is an inflammation-associated disease, the present study aimed to evaluate the effect of simvastatin on Runx-2, Osterix, and interleukin (IL) 17, 22 and 23 gene expression in ligature-induced periodontitis in rats.

METHODS

Wistar rats (300 g) were obtained from the Centro Multidisciplinar para Investigação Biológica na Área da Ciência em Animais de Laboratório (Campinas, Brazil), and received food and water *ad libitum*. The experiments were approved by the Ethics Committee of the University of Campinas (#3800-1) and followed in agreement with the Ethical Principles for Animal Research.

The rats were randomly distributed into two groups (saline and simvastatin) of ten animals each with a completely randomized split-mouth design. The contralateral molars were used as the unligated control in both groups. For induction of experimental periodontitis, the animals were anaesthetized with a single intra-muscular injection of ketamine (90 mg/kg; Dopalen, Ceva, Brazil) and xylazine (10 mg/kg; Rompun, Bayer, Brazil). Afterward, a cotton ligature (Gutterman®) was placed in a subgingival position encircling the entire cervix of the lower right first molars, which acted as a gingival irritating agent and promoted bacterial plaque accumulation [13]. The contralateral molars were left without a ligature in both groups and was used as control. Twenty-four hours after placement of the ligature, 25 mg/kg of saline solution dissolved in simvastatin (STG, Sigma-Aldrich St. Louis, MO, USA), and 10 ml/Kg of NaCl 0.9% saline solution (vehicle, VCG) was daily orally administered with a syringe, for 14 days.

After, the animals were euthanized by anesthetic overdose. Gingival tissue and bone samples from the molar region from both sides, were collected and immediately kept at-80ºC.

The RNA was extracted from bone and gingival tissues with TRIzol Reagent (Life Technologies) and subsequently submitted to reverse transcription using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific), according to the manufacturer's instructions. Quantitative (q) PCR was performed using a 7500 Fast Real Time PCR System (Thermo Scientific) with the Maxima SYBR Green qPCR Master Mix (Thermo Scientific). Cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minutes. The primer sets are listed in table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as internal gene reference. The quantification data were analyzed with the SDS System Software, and the relative expression levels were calculated according to the comparative Ct method, as $2^{-\Delta\Delta Ct}$.

Table 1. Genes analyzed and primer sequences.

Protein	Gene	Primer sequences
Runt-related transcription factor 2	*Runx2*	F 5'-AACCCACCCAGTAGCAAACC-3' R 5'-GGCATCAGACAAACACACGG-3'
Osterix	*Sp7*	F 5'-GGTCCTGGCAACACTCCTAC-3' R 5'-GGCATCAGACAAACACACGG-3'
Interleukin 17	*Il17a*	F 5'-CTACCTCAACCGTTCCACTTCAC-3' R 5'-CCTCCCAGATCACACAGAAGGATATCT-3'
Interleukin 22	*Il22*	F 5'-ACTTCCAGCAGCCATACATCGT-3' R 5'-AGCCTGACATCTGTCGTTGTTATCTG-3'
Interleukin 23	*Il23a*	F 5'-GACCAGCTTCATACCTCCCTACTG-3' R 5'-AGGCGAGGCATCTGTTGAGT-3'
Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	F 5'-TGGCCTCCAAGGAGTAAGAAAC-3' R 5'-TGGAAATTGTGAGGGAGATGCT-3'

Note: F: Forward; R: Reverse.

All experiments were performed in technical triplicates. The data were subjected to Mann-Whitney test, using SigmaPlot for Windows software, version 11 (Systat Software Inc., San Jose, CA), and the level of significance was set at 5%.

RESULTS

In bone tissue, the results evidenced that the use of simvastatin did not affect the Runx-2 expression when compared to the control group (figure 1A, $p>0.05$). However, a significant increase in the levels of Osterix transcripts after simvastatin exposure was observed when compared to control group ($p<0.05$) (figure 1B).

In the gingival samples, simvastatin reduced the IL-17, IL-22 and IL-23 gene expression levels, in comparison those identified in the control group ($p<0.05$) (figure 1C-E).

DISCUSSION

In the present study was evaluate the effects of simvastatin on Runx-2, Osterix, IL-17, IL-22, and IL-23 gene expression in ligature-induced periodontitis in rats. The results indicated that simvastatin did not affect the expression of Runx-2, but enhanced Osterix gene expression. On the other hand, the gene expression of all proinflammatory interleukins assayed was modulated negatively by simvastatin. Taken together, the results suggest that simvastatin may modified the path of the periodontal disease reinforcing its effect as an immunomodulatory drug.

Although many *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory properties of simvastatin as well as its effect on inhibiting matrix metalloproteases and bone resorption on periodontal disease [7,15,16], this study has been conducted to comprehend the effects of simvastatin on hard and soft tissues, separately, by means of measurement of transcripts for both genes encoding transcriptions factors and inflammatory interleukins in bone and gingival tissues, respectively.

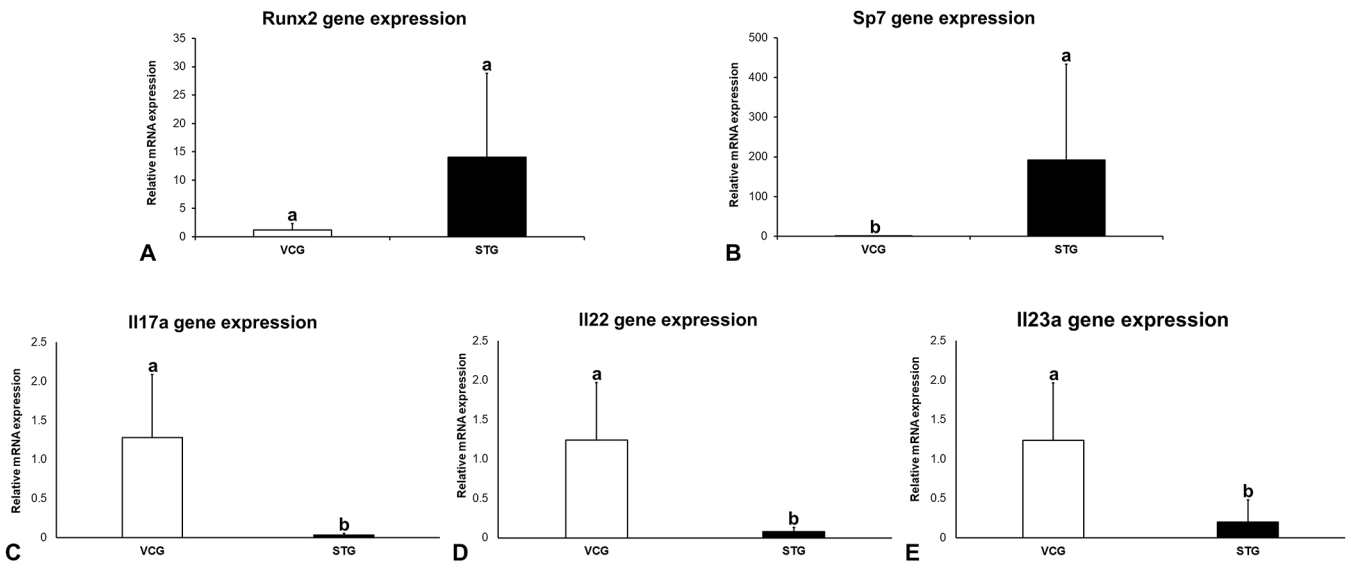

Figure 1. Quantification of Runx-2 (Runx2), Osterix (Sp7), IL-17 (Il17a), IL-22 (Il22), and IL-23 (Il23a) in in ligature-induced periodontitis in an animal model treated (STG) or not with simvastatin (VCG).
Note: Different letters indicate statistical significance among the groups for each time points (p<0.05). Different letters indicate statistical significance (p<0.05). Data are presented as mean and standard deviation.

The dose and administration of the simvastatin promoting benefits in local tissue relating to periodontal disease, remains unclear. Although there is no consensus regarding the optimal statin dose as anti-inflammatory adjuvant therapy, doses ranging from 1 to 35 mg/kg/day have been applied in some studies [7,17-19] and have resulted in controversial effects, especially on bone tissue. In this study, 25 mg/Kg simvastatin was systemically administered after periodontal disease induction for 14 days, as previously demonstrated by Mouchrek et al. [13].

Although many studies have demonstrated the beneficial effect from local statin application [16,20], our study evidenced that the systemic statin administration seems to provide some positive additional outcome on modulate the inflammatory process.

The anabolic action of this drug in recovering PD-related alveolar bone loss has already been studied. Indeed, simvastatin stimulates bone function, by increasing BMP-2 expression [14,21], as well as the release of the vascular endothelial growth factor, osteocalcin and bone sialoprotein [21,22]. These data reinforce the results of the present study, where the Osterix gene expression was upregulated after simvastatin administration. Osterix is a transcript factor that induces a range of genes involved to osteoblast differentiation, such as type I collagen, osteonectin, osteopontin, osteocalcin, and bone sialoprotein which are all essential for osteoblasts calcified bone matrix formation [23]. Runx-2 is another transcription factor important that can orchestrate bone formation along Osterix, promoting bone formation and osteoblast differentiation [24]. Despite no significant difference was observed, a notable tendency towards Runx-2 gene expression upregulation after simvastatin administration, highlighting their potential on bone protective responses under microbiological periodontal challenge.

Besides inflammatory cell infiltration, the periodontal disease is marked by high levels of a myriad cytokines and enzyme proteases leading to the degradation of inserting periodontium [25]. Among them, the interleukins (IL) exert an important role in the periodontitis disease pathogenesis. IL-17 is the most

pro-inflammatory explored cytokine and exerts an important role in several inflammatory conditions [26]. This cytokine is secreted especially by T helper 17 lineage [27], which is strongly associated with the capacity to induce osteoclastogenesis, caused by IL-17-mediated stimulation of the receptor activator of the NF-κB ligand (RANKL) on osteoblastic cells [28]. IL-22 and IL-23 cytokines are elevated in tissue samples and gingival crevicular fluid of patients with periodontal disease [29,30], playing an important role in inflammatory tissue destruction in periodontal lesions. In this study, simvastatin significantly reduced the levels of proinflammatory cytokines (IL-17, IL-22 and IL-23) in gingival tissues subjected to periodontitis, reinforcing its immunomodulatory effects, hindering the progression of the disease.

The present data demonstrated that the administration of simvastatin in a rodent model with ligature induced periodontitis significantly reduced Osterix transcription factor and inflammatory interleukins (IL-17, -22 and -23) gene expression contributing to avoid bone loss and inflammation promoted by periodontal disease. These findings may suggest, in part, some beneficial effects of simvastatin observed in statin-treated patients, which may alter the outcome of the disease, and consequently, the treatment approach.

Conflict of interest: The authors declare that there are no conflicts of interest.

Data availability: The research data are available from the corresponding author upon reasonable request.

Collaborators

AK Saba, conceptualization, investigation, methodology, project administration, supervision, validation, writing - original draft. LN Teixeira, conceptualization, methodology, validation, visualization, writing - original draft, writing - review and editing. E Saba-Chufji, investigation, writing- original draft. JCE Mouchrek Júnior, conceptualization, investigation, methodology, project administration, supervision, validation, writing-original draft. EF Martinez, conceptualization, investigation, methodology, project administration, supervision, validation, writing- original draft, writing - review and editing.

REFERENCES

1. Sanz M, van Winkelhoff AJ, Working Group 1 of Seventh European Workshop on Periodontology. Periodontal infections: understanding the complexity--consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011;38 Suppl 11:3-6. https://doi.org/10.1111/j.1600-051X.2010.01681.x
2. Yucel-Lindberg T, Båge T. Inflammatory mediators in the pathogenesis of periodontitis. Expert Rev Mol Med. 2013;15:e7. https://doi.org/10.1017/erm.2013.8
3. Bartold PM, Cantley MD, Haynes DR. Mechanisms and control of pathologic bone loss in periodontitis. Periodontol 2000. 2010;53:55-69. https://doi.org/10.1111/j.1600-0757.2010.00347.x
4. Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, et al. Association between periodontal pathogens and systemic disease. Biomed J. 2019;42(1):27-35. https://doi.org/10.1016/j.bj.2018.12.001
5. Zeiser R. Immune modulatory effects of statins. Immunology. 2018;154(1):69-75. https://doi.org/10.1111/imm.12902
6. Jadhav SB, Jain GK. Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol. 2006;58(1):3-18. https://doi.org/10.1211/jpp.58.1.0002
7. Dalcico R, Menezes AM, Deocleciano OB, Oriá RB, Vale ML, Ribeiro RA, et al. Protective mechanisms of simvastatin in experimental periodontal disease. J Periodontol. 2013;84(8):1145-57. https://doi.org/10.1902/jop.2012.120114
8. Liu M, Jin YH, Li TH, Shi LH, Zhu BQ. Effect of simvastatin on atherosclerosis and central aortic pressure in ApoE gene knockout mice. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2014;43(3):293-7. https://doi.org/10.3785/j.issn.1008-9292.2014.05.006
9. Pradeep AR, Karvekar S, Nagpal K, Patnaik K, Guruprasad CN, Kumaraswamy KM. Efficacy of locally delivered 1.2% rosuvastatin gel to non-surgical treatment of patients with chronic periodontitis: a randomized, placebo-controlled clinical trial. J Periodontol. 2015;86(6):738-45. https://doi.org/10.1902/jop.2015.140631
10. Bradley AD, Zhang Y, Jia Z, Zhao G, Wang X, Pranke L, et al. Effect of simvastatin prodrug on experimental periodontitis. J Periodontol. 2016;87(5):577-82. https://doi.org/10.1902/jop.2016.150599
11. Zhou J, Gao X, Huang S, Ma L, Cui Y, Wang H, et al. Simvastatin improves the jaw bone microstructural defect

induced by high cholesterol diet in rats by regulating autophagic flux. Biomed Res Int. 2018;2018:4147932. https://doi.org/10.1155/2018/4147932

12. Araújo Júnior RF, Souza TO, Moura LM, Torres KP, Souza LB, Alves Mdo S, et al. Atorvastatin decreases bone loss, inflammation and oxidative stress in experimental periodontitis. PLoS One. 2013;8(10):e75322. https://doi.org/10.1371/journal.pone.0075322
13. Mouchrek Júnior JCE, Macedo CG, Abdalla HB, Saba AK, Teixeira LN, Mouchrek AQES, et al. Simvastatin modulates gingival cytokine and MMP production in a rat model of ligature-induced periodontitis. Clin Cosmet Investig Dent. 2017;9:33-8. https://doi.org/10.2147/CCIDE.S134125
14. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. Science. 1999;286(5446):1946-9. https://doi.org/10.1126/science.286.5446.1946
15. Liu S, Bertl K, Sun H, Liu ZH, Andrukhov O, Rausch-Fan X. Effect of simvastatin on the osteogenetic behavior of alveolar osteoblasts and periodontal ligament cells. Hum Cell. 2012;25(2):29-35. https://doi.org/10.1007/s13577-011-0028-x
16. Bertl K, Steiner I, Pandis N, Buhlin K, Klinge B, Stavropoulos A. Statins in nonsurgical and surgical periodontal therapy. A systematic review and meta-analysis of preclinical in vivo trials. J Periodontal Res. 2018;53(3):267-87. https://doi.org/10.1111/jre.12514
17. Maritz FJ, Conradie MM, Hulley PA, Gopal R, Hough S. Effect of statins on bone mineral density and bone histomorphometry in rodents. Arterioscler Thromb Vasc Biol. 2001;21(10):1636-41. https://doi.org/10.1161/hq1001.097781
18. Nassar CA, Battistetti GD, Nahsan FP, Olegário J, Marconato J, Marin CF, et al. Evaluation of the effect of simvastatin on the progression of alveolar bone loss in experimental periodontitis: an animal study. J Int Acad Periodontol. 2014;16(1):2-7.
19. Xu XC, Chen H, Zhang X, Zhai ZJ, Liu XQ, Qin A, et al. Simvastatin prevents alveolar bone loss in an experimental rat model of periodontitis after ovariectomy. J Transl Med. 20141;12:284. https://doi.org/10.1186/s12967-014-0284-0
20. Bertl K, Parllaku A, Pandis N, Buhlin K, Klinge B, Stavropoulos A. The effect of local and systemic statin use as an adjunct to non-surgical and surgical periodontal therapy-A systematic review and meta-analysis. J Dent. 2017;67:18-28. https://doi.org/10.1016/j.jdent.2017.08.011.
21. Junqueira JC, Mancini MN, Carvalho YR, Anbinder AL, Balducci I, Rocha RF. Effects of simvastatin on bone regeneration in the mandibles of ovariectomized rats and on blood cholesterol levels. J Oral Sci. 2002;44(3-4):117-24. https://doi.org/10.2334/josnusd.44.117
22. Song C, Guo Z, Ma Q, Chen Z, Liu Z, Jia H, et al. Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. Biochem Biophys Res Commun. 2003;308(3):458-62. https://doi.org/10.1016/s0006-291x(03)01408-6
23. Renn J, Winkler C. Osterix-mCherry transgenic medaka for in vivo imaging of bone formation. Dev Dyn. 2009;238(1):241-8. https://doi.org/10.1002/dvdy.21836
24. Franceschi RT, Xiao G. Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. J Cell Biochem. 2003;88(3):446-54. https://doi.org/10.1002/jcb.10369
25. Loos BG, Tjoa S. Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? Periodontol 2000. 2005;39:53-72. https://doi.org/10.1111/j.1600-0757.2005.00129.x
26. Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. Cytokine. 2013;64(2):477-85. https://doi.org/10.1016/j.cyto.2013.07.022
27. Figueredo CM, Lira-Junior R, Love RM. T and B cells in periodontal disease: new functions in A Complex Scenario. Int J Mol Sci. 2019;20(16):3949. https://doi.org/10.3390/ijms20163949
28. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med. 2006;203(12):2673-82. https://doi.org/10.1084/jem.20061775
29. Ohyama H, Kato-Kogoe N, Kuhara A, Nishimura F, Nakasho K, Yamanegi K, et al. The involvement of IL-23 and the Th17 pathway in periodontitis. J Dent Res. 2009;88(7):633-8. https://doi.org/10.1177/0022034509339889
30. AlQranei MS, Chellaiah MA. Osteoclastogenesis in periodontal diseases: possible mediators and mechanisms. J Oral Biosci. 2020;62(2):123-30. https://doi.org/10.1016/j.job.2020.02.002

Received on: 7/11/2024
Approved on: 15/3/2025

Assistant editor: Luciana Butini Oliveira