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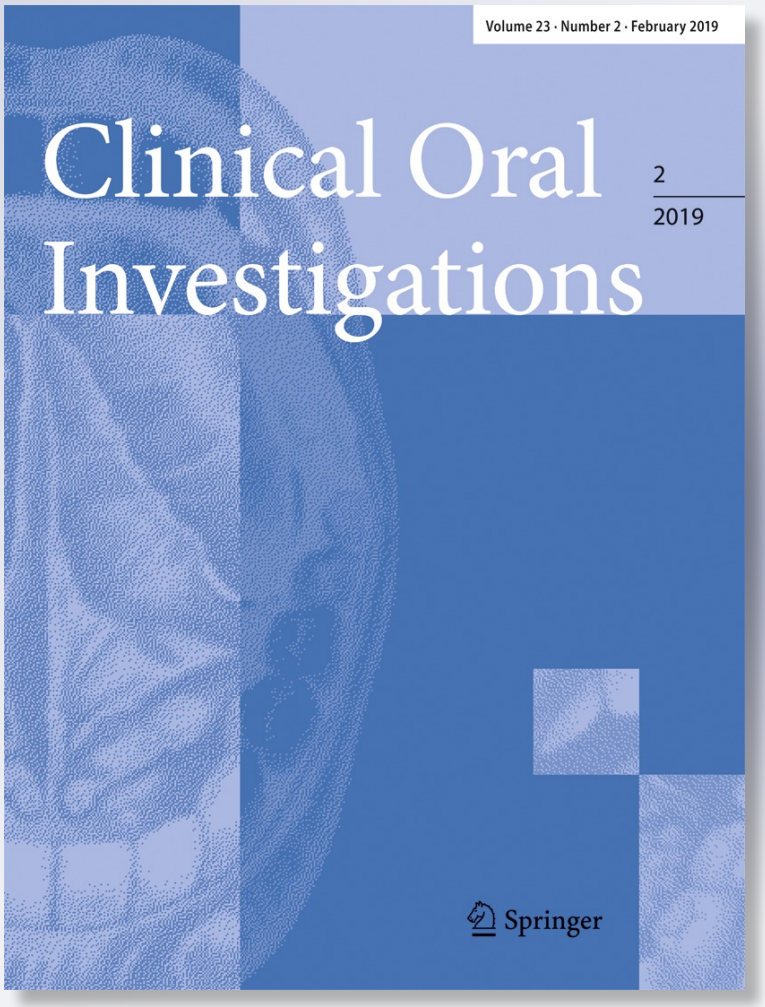

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ORIGINAL ARTICLE

Effect of intracanal medicaments on matrix metalloproteinase-9 and vasoactive intestinal peptide secretion in periapical lesions of re-treated canals: a randomized controlled clinical study

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Abstract

Objective The aim of this study was to evaluate the effect of calcium hydroxide ($Ca[OH]_2$) and chlorhexidine (CHX) gel on matrix metalloproteinase-9 (MMP-9) and vasoactive intestinal peptide (VIP) secretion in periapical lesions.

Materials and methods A total of 60 patients were randomly divided into two groups that were to receive different medications. Pre-and post-treatment samples were collected from the interstitial fluid of periapical lesions using sterile paper points. VIP and MMP-9 levels were measured by enzyme-linked immunosorbent assay kits, and the data were statistically analyzed.

Results Gender and smoking habits had no effect on the pre- and post-treatment VIP and MMPs levels. Intragroup analyses revealed that in the $Ca(OH)_2$ group, the post-treatment VIP level was found to be significantly higher than the pre-treatment VIP level. In the CHX group, the post-treatment MMP-9 level was significantly higher than the pre-treatment MMP-9 level.

Conclusion According to the results of the present study, the type of the medication affected the amount of periapical VIP and MMP-9 secretion.

Clinical relevance VIP is a neuropeptide that promotes new bone formation. Thus, intracanal $Ca(OH)_2$ medication may accelerate the repair process of bone tissue.

Keywords $Ca(OH)_2$ · CHX gel · Vasoactive intestinal peptide · Matrix metalloproteinase-9

Introduction

Apical periodontitis is an inflammatory disease of periapical tissues that is usually induced by a bacterial infection in the root canal system [1]. Bacteria and toxins in the root canal system activate a local immune response after reaching the periapical tissues through the apical foramen [1–3]. Several proinflammatory and immunoregulatory cytokines, mediators, chemokines, and neuropeptides are involved in this local immune response [1–3], during which these molecules degrade extracellular matrix (ECM) components, which are the main components of the connective tissue.

During an immune response, immune cells and dental pulp neurons release vasoactive intestinal peptide (VIP), which has potent immunomodulatory properties [4, 5]. VIP may regulate the growth of apical periodontitis lesions by inhibiting bone resorption through the suppression of osteoclast functions [6]. In addition, during an immune response, the immune cells release matrix metalloproteinases (MMPs) that degrade all ECM components, including the bone matrix [7, 8]. Several studies have shown that MMPs participate in the pathogenesis of pulp and periapical inflammation [9, 10].

Matrix metalloproteinase-9 (MMP-9) plays an important role in the development of periapical lesions and is highly expressed in apical periodontitis [11, 12]. A recent study suggested that the epigenetic modulation of the MMP-9 gene may contribute to the pathogenesis of periapical granulomas and radicular cysts [13]. Furthermore, a possible correlation of MMP-9 polymorphisms with chronic periodontitis has been reported [14].

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Because bacteria and their byproducts in the root canal system are the primary causes of apical periodontitis, it is important to develop an optimal root canal disinfection protocol [15]. Intracanal medicaments such as calcium hydroxide ($Ca[OH]_2$) [16] and chlorhexidine (CHX) gel [17] are widely used in root canal treatments because of their antimicrobial and anti-inflammatory properties. Although previous studies have evaluated the anti-inflammatory properties of $Ca(OH)_2$ [18] and CHX gel [19], no study has investigated the effects of these two intracanal medicaments on the secretion of VIP and MMP-9 in periapical lesions after a root canal treatment. This is a critical gap in the literature, because VIP and MMP-9 may play an important role in mediating the pathogenesis of periradicular periodontitis. Several studies have revealed that intracanal medication affects cytokine levels in apical periodontitis [20–22]. Therefore, it can be hypothesized that the level of VIP and MMP-9 secretion could be affected by the type of intracanal medication.The present study investigated the effects of these intracanal medicaments on MMP-9 and VIP secretion in periapical lesions. The null hypothesis of this study was that the use of $Ca(OH)_2$ paste and CHX gel for intracanal medication would not affect VIP and MMP-9 secretion levels in periapical lesions.

Material and methods

Patient selection

All study participants were selected from a pool of patients who were referred to the Department of Endodontics, Faculty of Dentistry, Atatürk University. The study protocol was approved by the ethical committee of the Faculty of Dentistry, Atatürk University (decision no. 2017-28). All the patients included in the study signed an informed consent form before undergoing the treatment. The sample size calculation, which was based on an error of $\alpha = 0.05$, a power of 0.8, and an effect size of 0.5, indicated that each group should include a minimum of 27 patients. Therefore, the study included a total of 60 patients (30 in each group).

Patients were included in the study if their teeth showed asymptomatic or chronic apical periodontitis, if they had previously undergone primary root canal treatment, if their teeth did not show any swelling, root fracture, ankyloses, or pathological mobility with a pocket depth < 3 mm, and if they had single-rooted teeth with one root canal. Exclusion criteria were treatment with antibiotics within 1 month prior to the study, the presence of internal or external resorption, and the presence of any systemic disease.

Treatment protocol

The study patients were randomly divided into two groups using a web program (www.randomizer.org), and the number of each patient and the number of groups were recorded. Before the treatment, the patients were administered epinephrine (dilution, 1:100,000) containing 1.8 ml of articaine HCl (Ultracaine DS Forte; Aventis, Istanbul, Turkey) to induce profound local anesthesia. The teeth of the patients were isolated using a rubber dam, and the crowns and surrounding structures of the teeth were disinfected using 30% H_2O_2 and 2.5% NaOCl for 30 s. Next, the effect of the NaOCl was removed using 5% sodium thiosulfate. An access cavity was opened under rubber dam isolation. Once root canal filling material was detected, the mass of gutta-percha was removed using a Reciproc R25 file (VDW, Munich, Germany). Working lengths were determined using an electronic apex locator (Raypex 6; VDW) with a 15-K hand file (Mani, Tochigi, Japan). Root canals were prepared using R25 and R50 Reciproc files at the determined working length. If gutta-percha was detected radiographically, further preparation was performed using large-sized K-files. During instrumentation, the root canals were irrigated using 2 ml of 1% NaOCl. Before sample collection, the root canals were irrigated with 5 ml of 1% NaOCl, 0.5% sodium thiosulfate, and distilled water. Three sterile paper points were inserted into the root canals through the root apex (2 mm) and were left in place for 1 min to sample the VIP and MMPs secreted into the interstitial fluid of apical tissues (s1) [21]. The paper points used for sample collection had a tip size of 0.020 mm. After being removed from the root canals, the paper points were cut 4 mm from the tip [21], and samples collected were stored in Eppendorf tubes containing phosphate-buffered saline at − 80 °C. Before intracanal medicament placement, the root canals were rinsed for the final time by using 1 ml of 17% EDTA (ENDO-SOLUTION; Cerkamed, Wojciech, Poland) following 5 ml distilled water for both groups (calcium hydroxide or chlorhexidine). Next, the root canals were dried using paper points, and $Ca(OH)_2$ paste (Calcicur; Voco, Cuxhaven, Germany) or CHX gel (Gluco-CHex 2% gel; Cerkamed) was placed into the canals at a distance of 1 mm less than the working length by using an EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK). Access cavities were sealed using Cavit-G (3M ESPE, Seefeld, Germany), and the patients were asked to re-visit the clinic 7 days after the initial appointment.

During the second visit, the root canals were aseptically opened under rubber dam isolation by using the same disinfection protocol described above. Intracanal medication was irrigated using 5 ml of distilled water and was mechanically removed using a master apical file. The root canals were irrigated again using 10 ml of saline solution. The $Ca(OH)_2$ was removed using 5 ml of 17% EDTA and distilled water with an EndoActivator, and the CHX gel was removed using 10 ml of distilled water with an EndoActivator. Samples were collected as described previously and were stored at − 80 °C for further analysis. Root canal obturations were performed using gutta-

Table 1 Distribution of patients according to smoking habbit, gender, and age

Parameter	Vasoactive ($n = 29$) $Ca(OH)_2$	Intestinal peptide CHX	P	Matrix ($n = 30$) $Ca(OH)_2$	Metalloproteinase-9 CHX	P
Mean age (years)	28 ± 8.7	28.4 ± 9.4	0.482	27.9 ± 8.5	29 ± 9.2	0.634
Gender						
Female	14	15	0.793	15	16	0.796
Male	15	14		15	14	
Smoking habit						
−	19	20	0.780	19	21	0.584
+	10	9		11	9	

− No smoking, + smoking

Ca(OH)₂ (calcium hydroxide), *CHX* (chlorhexidine gel)

percha and root canal sealers, and permanent restoration was performed after the obturation.

Quantification of VIP and MMP-9 secretion levels

VIP and MMP-9 levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits (for VIP: Eastbiopharm, Hangzhou, China, Cat. No. CK-E90319; for MMP-9: Affymetrix, eBioscience, San Diego, CA, USA, Cat. No. BMS2016/2), according to the manufacturers' instructions. For the ELISA assays, the sample solutions did not receive any specific treatment. The solution did not lyophilize because there were not enough particles in the solution to initiate the lyophilization procedure.

Standard and sample solutions were added to 96-well plates precoated with specific anti-VIP and anti-MMP-9 antibodies. Next, biotin-conjugated anti-human antibodies were added to the wells. After incubation, the unbound biotin-conjugated anti-human antibodies were removed by washing the wells. Next, streptavidin-horseradish peroxidase (HRP), which binds to biotin-conjugated anti-human VIP and MMP-9 antibodies, was added to the wells, and the wells were incubated for 60 min at room temperature. After washing to remove unbound streptavidin-HRP, a substrate solution was added to the wells. Finally, VIP and MMP-9 levels were determined using an ELISA reader at an absorbance of 450 nm. Tests for each biomarker were carried out in duplicate. The results of the measurements were estimated using the standard curves included in the VIP and MMP-9 assay kits.

Statistical analysis

The study data were analyzed using IBM® SPSS® Statistics 20 software (IBM SPSS Inc., Chicago, IL, USA). A generalized linear model analysis was performed to determine the most effective factor (treatment group, sex, and smoking status) associated with the difference in pre- and post-treatment MMP-9 and VIP levels. For intra-group analysis, the Wilcoxon test was used to compare pre- and post-treatment VIP and MMP-9 levels because the data obtained were not normally distributed. For inter-group analysis, the Mann-Whitney U test was used to compare the percentage changes between pre- and post-treatment MMP-9 and VIP values. Data on gender and smoking status were analyzed using the chi-square test. The significance value for all tests was set at 5% ($P = 0.05$).

Results

A total of 60 patients who were referred to the Department of Endodontics, Faculty of Dentistry at Atatürk University were included in the study. The preoperative demographic features according to the patient groups are displayed in Table 1 below. Statistical analysis demonstrated no significant differences between the groups in terms of gender, smoking habit, or age ($P > 0.05$).

According to the generalized linear model analyses, the pre- and post-treatment VIP levels, which were chosen as

Table 2 Generalized Linear Model findings for gender, smoking habit, and type of intracanal medication (group) on the dependent variable "VIP and MMP-9 secretion level"

	Vasoactive Std. error	İntestinal Beta	Peptide P value	Matrix Std. Error	Metalloproteinase-9 Beta	P value
Gender	299.225	0.195	0.133	1.668	− .131	.333
Smoking habit	318.803	− 0.076	0.551	1.771	.164	.229
Group	294.333	0.337	0.010*	1.624	.132	.315

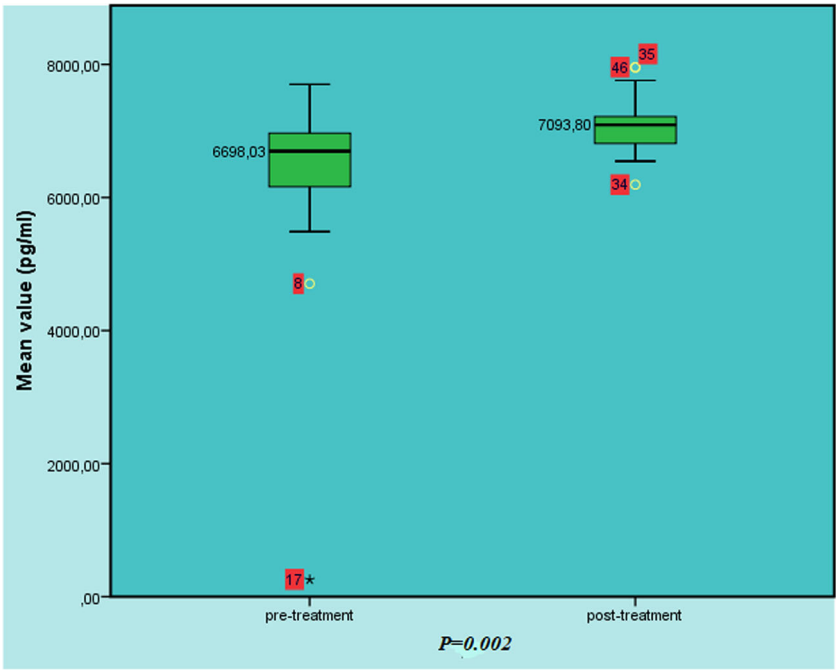

Fig. 1 Box plots show mean value of pre- and post-treatment VIP levels for $Ca(OH)_2$ group

the dependent variable, were affected by the type of intracanal medication ($P < 0.05$). However, the pre- and post-treatment MMPs levels, which were chosen as the dependent variable, were not affected by the type of medication ($P > 0.05$). Gender and smoking habits had no effect on either pre- or post-treatment levels of VIP or MMPs ($P > 0.05$) (Table 2).

Pre- and post-treatment levels of VIP and MMPs for the $Ca(OH)_2$ and CHX groups are shown in Figs. 1, 2, 3, and 4. Intragroup analyses revealed that, in the $Ca(OH)_2$ group, post-treatment VIP levels were significantly higher than pre-treatment VIP levels ($P < 0.05$). However, in the CHX group, there was no statistically significant difference between the pre- and post-treatment VIP levels ($P > 0.05$). Conversely, in the CHX group, the post-treatment level of MMP-9 was significantly higher than the pre-treatment level ($P < 0.05$), while in the $Ca(OH)_2$ group, there was no statistically significant difference between the pre- and post-treatment levels of MMP-9 ($P > 0.05$).

Intergroup analyses revealed that there was no statistically significant difference between the $Ca(OH)_2$ and CHX groups in the preoperative and postoperative percentage change in

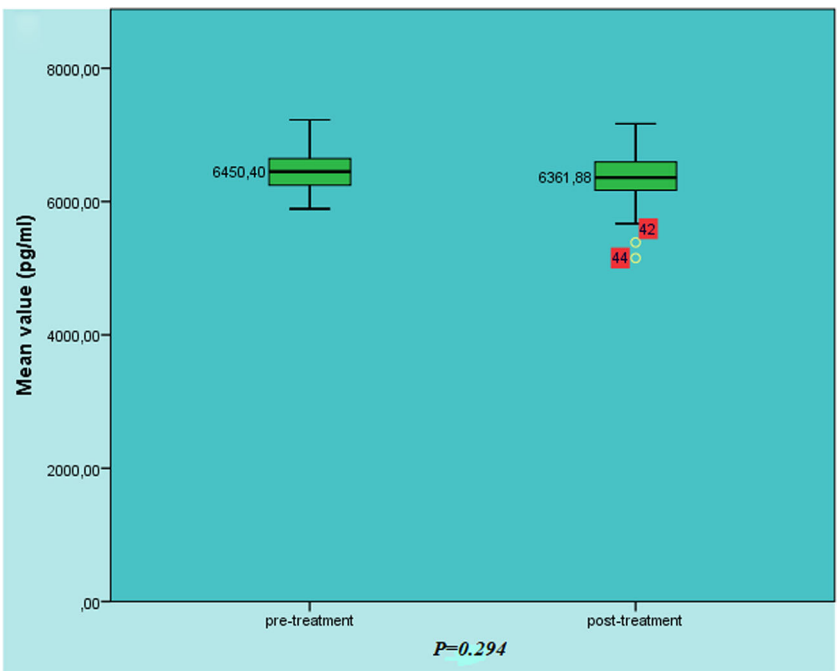

Fig. 2 Box plots show mean value of pre- and post-treatment VIP levels for CHX group

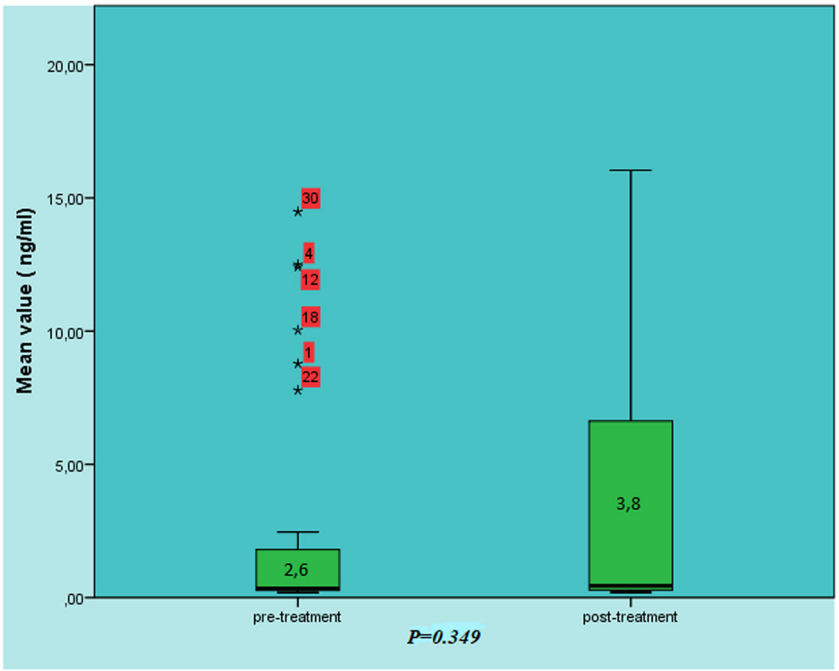

Fig. 3 Box plots show mean value of pre- and post-treatment MMP 9 levels for $Ca(OH)_2$ group

MMP-9 values. However, there was a statistically significant difference between the $Ca(OH)_2$ and CHX groups in the preoperative and postoperative percentage change in VIP values (Figs. 5 and 6).

Discussion

During periapical inflammation, host cells in the periapical tissues release several cytokines, chemokines, leukotrienes, prostaglandins, and proteolytic enzymes into the periapical area [1]. MMPs are proteolytic enzymes produced by neutrophil granulocytes, macrophages, eosinophils, and T cells [23] and may play an important role in the degradation of the ECM, leading to the development of periapical lesions [24]. In contrast, VIP, a neuropeptide, reduces inflammatory response [25] and inhibits bone resorption by regulating osteoclast maturation via inducing the expression of key proteins associated with osteoclast differentiation [6]. The results of the present study indicate that the type of medication affected

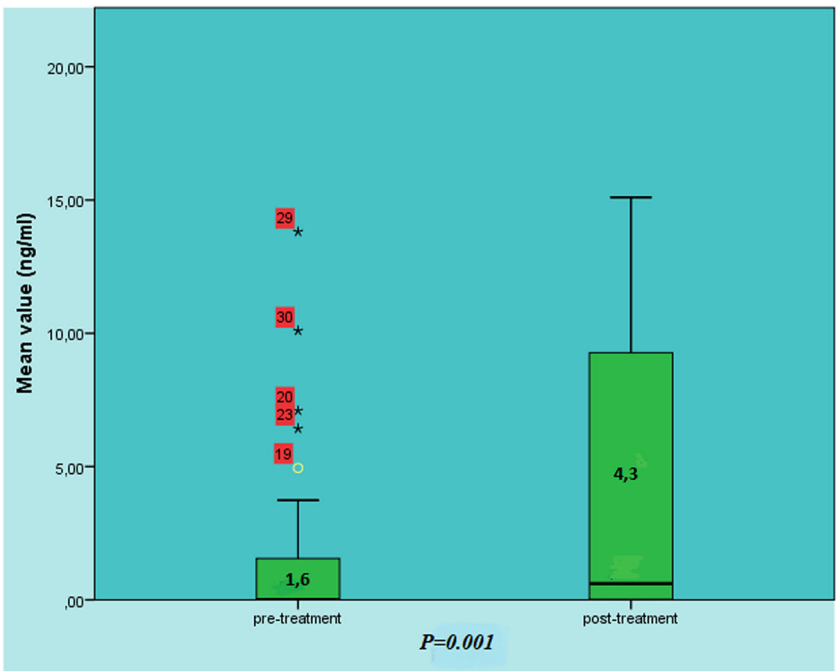

Fig. 4 Box plots show mean value of pre- and post-treatment MMP 9 levels for CHX group

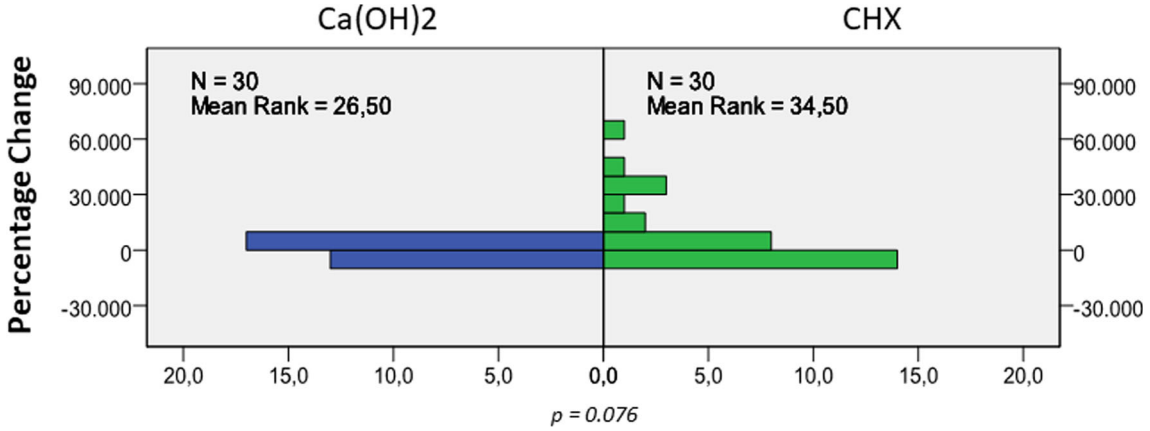

Fig. 5 Preoperative-postoperative percentage change of MMP 9 levels for $Ca(OH)_2$ and CHX groups

MMP-9 and VIP secretion levels, thus rejecting the null hypothesis.

The results of the present study showed no significant differences between pre- and post-treatment MMP-9 levels in patients in the $Ca(OH)_2$ group. Because no study has evaluated the effect of $Ca(OH)_2$ on MMP-9 secretion levels, we cannot directly compare our results with those of previous studies. However, several in vivo and in vitro studies have evaluated the effect of $Ca(OH)_2$ on bacterial lipopolysaccharide (LPS) [26, 27] and reported that $Ca(OH)_2$ lowers endotoxin concentration. Martinho et al. [28] showed that increased endotoxin levels in root canals were positively correlated with high MMP-9 levels. In the present study, intracanal treatment with $Ca(OH)_2$ may have prevented the increase in MMP-9 levels in periapical lesions because of its possible detoxifying effect on LPS.

The results of the present study have shown that post-treatment VIP levels were significantly higher than pre-treatment VIP levels in patients in the $Ca(OH)_2$ group. VIP expression levels are inversely correlated with the diameter of chronic periradicular lesions [29]; VIP levels are higher in periapical lesions with a small diameter. The ability of $Ca(OH)_2$ to reduce osteoclast-like cell differentiation [30] may have shifted these lesions to the healing stage and increased VIP levels. Moreover, $Ca(OH)_2$ increases Th-2-type cytokine levels [21], and the stimulation of Th-2-type cytokines induces VIP expression [31]. However, there are several factors that influence the immune response. Therefore, it is more relevant to conclude that the medications were effective in reducing intra-canal bacteria, and the host immune response subsequently changed.

The results of the present study showed that post-treatment MMP-9 levels were significantly higher than pre-treatment MMP-9 levels in patients in the CHX group. CHX is a water-soluble molecule with a pH between 5 and 7 [32] and has a minimal effect on LPS [33]. Kato et al. [34] reported an inverse correlation between MMP-9 secretion levels and pH level. Specifically, they reported that MMP-9 secretion levels were higher at a pH of 6.8 than at a pH of 7.3. The neutral pH of CHX and its ineffectiveness against LPS may have produced an acidic environment in the periapical region, thereby increasing MMP-9 levels. However, no significant difference was observed between pre- and post-treatment VIP levels in patients in the CHX group. This may be because of the minimal effect of the CHX gel on Th-2-type cytokine response [21] and because VIP expression is positively correlated with the stimulation of Th-2-type cells [33].

The present study evaluated MMP-9 because several studies have shown that MMP-9 is a multidomain enzyme that

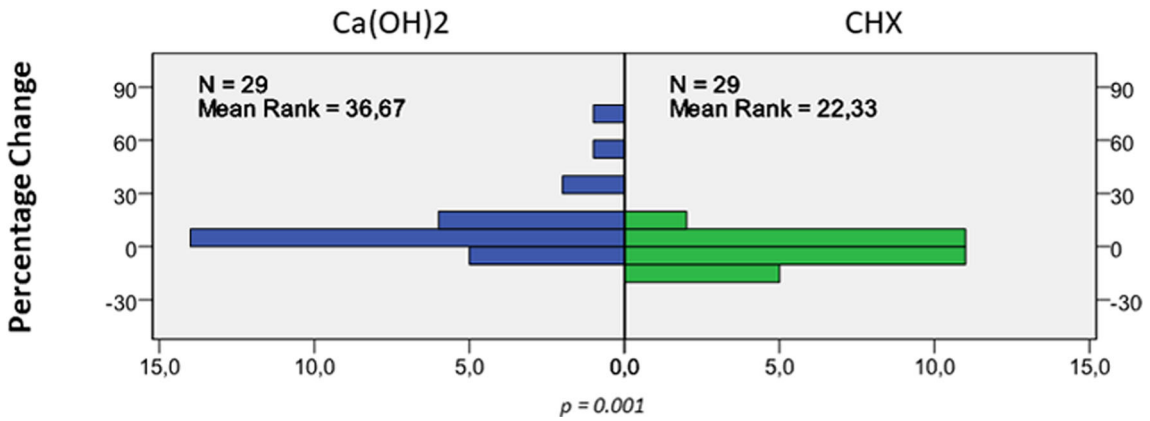

Fig. 6 Preoperative-postoperative percentage change of VIP levels for $Ca(OH)_2$ and CHX groups

plays an important role in acute and chronic inflammatory diseases [35] and in the development of periapical lesions by inducing osteoclastic resorption, probably by degrading the ECM [36]. MMP-9 expression is high in periapical lesions, suggesting a significant role in the pathogenesis of apical periodontitis [13].

In the present study, periapical samples were collected using a method similar to that reported by Martinho et al. [21]. EDTA was used to remove $Ca(OH)_2$ because a previous study reported that EDTA is effective at removing $Ca(OH)_2$ from root canals [37]. EDTA reacts with calcium ions and forms soluble calcium chelates [38], subsequently becoming easier to remove from root canal walls [37, 39]. The chelating effect of EDTA is self-limiting because equilibrium is formed when all ions have been bound [40]. In addition, EDTA has minimal antimicrobial effects [41]. It was previously reported that EDTA has no significant effect on biofilm viability [42] and was ineffective against E. faecalis even after 60 min of contact [43]. In the present study, a 1-min EDTA application was used to remove the Ca(OH)2. Therefore, it is unlikely that removing the Ca(OH)2 using EDTA interfered with the results of the Ca(OH)2 group. However, the CHX was removed using distilled water because CHX easily dissolves in water [32].

In the present study, 120 samples were collected for all groups (60 preoperative and 60 postoperative). For each sample, the paper points were added to a 120-μl solution, so 100 μl of sample were obtained from each tooth. Then, the 100-μl solution was divided into two portions because the lowest volume needed for an analysis was 50 μl. For each sample, the absorbance was measured twice, and the mean of the two measurements was used for the statistical analysis. Consequently, 240 measurements (120 preoperative and 120 postoperative) were performed for all groups to increase the statistical power.

According to the results of the present study, the type of medication affected the amount of periapical VIP and MMP-9 secretion. VIP is a neuropeptide that promotes new bone formation, and the present study was the first to reveal that the level of VIP secretion in periapical lesions increases when Ca(OH)2 is used for intracanal medication between treatment visits. Further research is needed to investigate the effect of intracanal medication with $Ca(OH)_2$ and CHX on the level of cytokine expression in periapical lesions.

Acknowledgements Manuscript in Lieu of Thesis

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the ethical committee of the Faculty of Dentistry, Atatürk University (decision no. 2017-28).

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Nair PN (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 15(6):348–381
2. Marton IJ, Kiss C (2014) Overlapping protective and destructive regulatory pathways in apical periodontitis. J Endod 40(2):155–163
3. Colic M et al (2006) Correlation between phenotypic characteristics of mononuclear cells isolated from human periapical lesions and their in vitro production of Th1 and Th2 cytokines. Arch Oral Biol 51(12):1120–1130
4. De la Fuente M, Delgado M, Gomariz RP (1996) VIP modulation of immune cell functions. Adv Neuroimmunol 6(1):75–91
5. Uddman R, Björlin G, Möller B, Sundler F (1980) Occurrence of VIP nerves in mammalian dental pulps. Acta Odontol Scand 38(5): 325–328
6. Lundberg P, Lie A, Bjurholm A, Lehenkari PP, Horton MA, Lerner UH, Ransjö M (2000) Vasoactive intestinal peptide regulates osteoclast activity via specific binding sites on both osteoclasts and osteoblasts. Bone 27(6):803–810
7. Mauviel A (1993) Cytokine regulation of metalloproteinase gene expression. J Cell Biochem 53(4):288–295
8. Bosman FT, Stamenkovic I (2003) Functional structure and composition of the extracellular matrix. J Pathol 200(4):423–428
9. Marton IJ, Kiss C (2000) Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol 15(3):139–150
10. Lin LM, Huang GT, Rosenberg PA (2007) Proliferation of epithelial cell rests, formation of apical cysts, and regression of apical cysts after periapical wound healing. J Endod 33(8):908–916
11. Carneiro E, Menezes R, Garlet GP, Garcia RB, Monteiro Bramante C, Figueira R, Sogayar M, Mauro Granjeiro J (2009) Expression analysis of matrix metalloproteinase-9 in epithelialized and nonepithelialized apical periodontitis lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107(1):127–132
12. Pereira Faustino IS, Azevedo RS, Takahama A Jr (2016) Metalloproteinases 2 and 9 immunoexpression in periapical lesions from primary endodontic infection: possible relationship with the histopathological diagnosis and the presence of pain. J Endod 42(4):547–551
13. Campos K, Gomes CC, Farias LC, Silva RM, Letra A, Gomez RS (2016) DNA methylation of MMP9 is associated with high levels of MMP-9 messenger RNA in periapical inflammatory lesions. J Endod 42(1):127–130
14. Ma T et al (2017) Correlation of matrix metalloproteinase-9 polymorphisms with chronic periodontitis in Uygur adults. Zhonghua Kou Qiang Yi Xue Za Zhi 52(6):360–366
15. Estrela C, Holland R, Estrela CRA, Alencar AHG, Sousa-Neto MD, Pécora JD (2014) Characterization of successful root canal treatment. Braz Dent J 25(1):3–11
16. Portenier I et al (2005) The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. J Endod 31(5):380–386
17. Lakhani AA et al (2017) Efficacy of triple antibiotic paste, moxifloxacin, calcium hydroxide and 2% chlorhexidine gel in elimination of E. Faecalis: an in vitro study. J Clin Diagn Res 11(1):ZC06–ZC09

18. Martinho FC et al (2017) Clinical comparison of the effectiveness of 7- and 14-day intracanal medications in root canal disinfection and inflammatory cytokines. Clin Oral Investig 22:523–530
19. Gendron R et al (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, *and 9 by chlorhexidine*. Clin Diagn Lab Immunol 6(3):437–439
20. Khan AA, Sun X, Hargreaves KM (2008) Effect of calcium hydroxide on proinflammatory cytokines and neuropeptides. J Endod 34(11):1360–1363
21. Martinho FC, Nascimento GG, Leite FRM, Gomes APM, Freitas LF, Camões ICG (2015) Clinical influence of different intracanal medications on Th1-type and Th2-type cytokine responses in apical periodontitis. J Endod 41(2):169–175
22. Paula-Silva FW, da Silva LA, Kapila YL (2010) Matrix metalloproteinase expression in teeth with apical periodontitis is differentially modulated by the modality of root canal treatment. J Endod 36(2): 231–237
23. Goetzl EJ, Banda MJ, Leppert D (1996) Matrix metalloproteinases in immunity. J Immunol 156(1):1–4
24. Corotti MV, Zambuzzi WF, Paiva KBS, Menezes R, Pinto LC, Lara VS, Granjeiro JM (2009) Immunolocalization of matrix metalloproteinases-2 and -9 during apical periodontitis development. Arch Oral Biol 54(8):764–771
25. Pozo D, Delgado M, Martínez C, Guerrero JM, Leceta J, Gomariz RP, Calvo JR (2000) Immunobiology of vasoactive intestinal peptide (VIP). Immunol Today 21(1):7–11
26. Adl A, Motamedifar M, Shams MS, Mirzaie A (2015) Clinical investigation of the effect of calcium hydroxide intracanal dressing on bacterial lipopolysaccharide reduction from infected root canals. Aust Endod J 41(1):12–16
27. Tanomaru JM et al (2003) Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J 36(11):733–739
28. Martinho FC, Teixeira FFC, Cardoso FGR, Ferreira NS, Nascimento GG, Carvalho CAT, Valera MC (2016) Clinical investigation of matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, and matrix metalloproteinase/tissue inhibitors of matrix metalloproteinase complexes and their networks in apical periodontitis. J Endod 42(7):1082–1088
29. Caviedes-Bucheli J, Azuero-Holguín MM, Moreno GC, González IL, Mateu E, Salazar JF, Munoz HR (2007) Vasoactive intestinal peptide receptor expression in chronic periapical lesions. Int Endod J 40(7):521–525
30. Jiang J, Zuo J, Chen SH, Holliday LS (2003) Calcium hydroxide reduces lipopolysaccharide-stimulated osteoclast formation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95(3):348–354
31. Vassiliou E, Jiang X, Delgado M, Ganea D (2001) TH2 lymphocytes secrete functional VIP upon antigen stimulation. Arch Physiol Biochem 109(4):365–368
32. Ferraz CC et al (2001) In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod 27(7):452–455
33. Buck RA et al (2001) Detoxification of endotoxin by endodontic irrigants and calcium hydroxide. J Endod 27(5):325–327
34. Kato Y, Nakayama Y, Umeda M, Miyazaki K (1992) Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. J Biol Chem 267(16):11424–11430
35. Zehnder M, Wegehaupt FJ, Attin T (2011) A first study on the usefulness of matrix metalloproteinase 9 from dentinal fluid to indicate pulp inflammation. J Endod 37(1):17–20
36. Manicone AM, McGuire JK (2008) Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 19(1):34–41
37. Rodig T et al (2010) Efficacy of different irrigants in the removal of calcium hydroxide from root canals. Int Endod J 43(6):519–527
38. Violich DR, Chandler NP (2010) The smear layer in endodontics—a review. Int Endod J 43(1):2–15
39. Chockattu SJ, Deepak BS, Goud KM (2017) Comparison of efficiency of ethylenediaminetetraacetic acid, citric acid, and etidronate in the removal of calcium hydroxide intracanal medicament using scanning electron microscopic analysis: an in-vitro study. J Conserv Dent 20(1):6–11
40. Hulsmann M, Heckendorff M, Lennon A (2003) Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 36(12):810–830
41. Chandra SS, Miglani R, Srinivasan MR, Indira R (2010) Antifungal efficacy of 5.25% sodium hypochlorite, 2% chlorhexidine gluconate, and 17% EDTA with and without an antifungal agent. J Endod 36(4):675–678
42. Ordinola-Zapata R, Bramante CM, Cavenago B, Graeff MSZ, Gomes de Moraes I, Marciano M, Duarte MAH (2012) Antimicrobial effect of endodontic solutions used as final irrigants on a dentine biofilm model. Int Endod J 45(2):162–168
43. Arias-Moliz MT, Ferrer-Luque CM, Espigares-Rodríguez E, Liébana-Ureña J, Espigares-García M (2008) Bactericidal activity of phosphoric acid, citric acid, and EDTA solutions against Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 106(2):e84–e89