ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta stomatol Croat. 2025;59(4):378-388.
DOI: 10.15644/asc59/4/5

ORIGINAL SCIENTIFIC PAPER
IZVORNI ZNANSTVENI RAD

Dobroslav Purešević[1], Ema Krdžovic Lazić[1,2], Nađa Nikolić[1], Ognjan Pavlović[1], Francesca Ideo[3], Miroslav Andrić[4], Maja Miletić[5], Aleksandar Jakovljević[1,5], Katarina Beljić-Ivanović[2]

Cross-Sectional Analysis of Matrix Metalloproteinases and Bone Resorption Regulators in Apical Periodontitis: Linking Molecular Mechanisms with Clinical and Histopathological Features

Presječna analiza razine matričnih metaloproteinaza i regulatora resorpcije kosti u apikalnom parodontitisu: povezivanje molekularnih mehanizama s kliničkim i histopatološkim značajkama

[1] Scientific Laboratories, Implant-Research Center, School of Dental Medicine, University of Belgrade, Serbia
Istraživački laboratoriji Implantološko-istraživačkoga centra, Stomatološki fakultet, Sveučilište u Beogradu, Srbija
[2] Department for Restorative Odontology and Endodontics, School of Dental Medicine, University of Belgrade, Serbia
Klinika za bolesti zuba, Stomatološki fakultet, Sveučilište u Beogradu, Srbija
[3] Department of Conservative Dentistry and Endodontics, University of Cagliari, Cagliari, Italy
Klinika za konzervativnu stomatologiju i endodonciju, Sveučilište u Cagliariju, Italija
[4] Department of Oral Surgery, School of Dental Medicine, University of Belgrade, Serbia
Klinika za oralnu hirurgiju, Stomatološki fakultet, Sveučilište u Beogradu, Srbija
[5] Department of Pathophysiology, School of Dental Medicine, University of Belgrade, Serbia
Odsjek za patološku fiziologiju, Stomatološki fakultet, Sveučilište u Beogradu, Srbija

Abstract

Objective(s) : (a) To quantify the relative gene expression of matrix metalloproteinases (MMP) *-2*, *-9*, their tissue inhibitor (TIMP) *-2*, receptor activator of nuclear factor kappa-B ligand *(RANKL)*, and osteoprotegerin *(OPG)* in human apical periodontitis (AP) and to associate it with levels of these molecules in healthy dental pulps. (b) To assess differences in gene expression among AP lesions stratified by clinical, radiographic, and histopathological features. (c) Finally, a potential correlation of investigated molecules was explored in AP samples. **Materials and Methods:** The study cohort comprised 80 AP lesions obtained from 80 adult volunteers during apicoectomy procedures. The healthy control cohort consisted of 50 dental pulp samples from intact teeth extracted for orthodontic purposes, collected from 50 voluntary donors. Relative gene expression of *MMP-2, MMP-9, TIMP-2, RANKL,* and *OPG* was assessed in all tissue samples using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). Statistical analyses included the Chi-square test, Student's t-test, Mann–Whitney U test, and Spearman correlation. **Results:** Higher gene expression levels of *RANKL, OPG, MMP-2,* and *MMP-9* were observed in the study compared to the healthy cohort (p=0.048, p=0.002, p=0.048, and p=0.012, respectively). Higher relative expression of the *MMP2* gene was observed in smaller lesions compared to large lesions (p=0.021). Significant positive correlations were observed between the investigated mediators. **Conclusions:** Concurrent overexpression of the studied mediators, along with their positive correlations in AP lesions, indicates their coordinated role in disease progression and alveolar bone resorption.

Received: August 28, 2025
Accepted: October 3, 2025

Address for correspondence
Aleksandar Jakovljevic, DDS, PhD
Department of Pathophysiology,
Scientific Laboratories, Implant-Research Center
School of Dental Medicine, University of Belgrade
Serbia, Dr Subotica Street, no.8,
11000 Belgrade, Serbia
Phone: +381112685288
a.jakovljevic@stomf.bg.ac.rs

MeSH Terms: Periapical Periodontitis; Inflammation Mediators; Matrix Metalloproteinases; Bone Resorption; Alveolar Bone Loss
Author Keywords: Apical Periodontitis; Inflammatory mediators, Matrix Metalloproteinases; Bone Resorption.

Dobroslav Puresevic – 009-0007-7431-0588
Ema Krdzovic Lazic - 0000-0002-6865-6423)
Nadja Nikolic - 0000-0002-8513-9313
Ognjan Pavlovic - 0000-0002-7897-8717
Francesca Ideo - 0000-0002-7116-0656
Miroslav Andric - 0000-0003-4136-7901
Maja Miletic - 0000-0002-8622-7171
Aleksandar Jakovljevic - 0000-0001-6512-4934
Katarina Beljic Ivanovic - 0000-0001-9169-5415

Introduction

Apical periodontitis (AP) is a chronic immune-inflammatory disorder that directly impacts the periapical tissues of the affected tooth (1). It is initiated as a host response to polymicrobial invasion of the dental pulp. This condition is fundamentally driven by bacterial colonization within the root canal system, which includes the formation of biofilms and the release of virulence factors. These factors, along with genetic susceptibility and other predisposing factors, decisively trigger an array of immune responses in the host (2 – 6). Therefore, the pathogenesis of AP is driven by a complex and multifaceted network integrating immune system activation, recruitment of various immune cells (e.g., phagocytes and lymphocytes), and production of different inflammation mediators [e.g., cytokines, chemokines, reactive oxygen species, matrix metalloproteinases (MMPs), bone resorption regulators, etc.] (7). These molecular mediators play a critical role in orchestrating the degradation of the extracellular matrix and the resorption of alveolar bone, which are central features in the progression of the lesions (8).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade collagen and other extracellular matrix (ECM) components (9 – 12). Their activity is regulated by tissue inhibitors of metalloproteinases (TIMPs) to prevent tissue damage. Under normal conditions, MMPs facilitate tissue remodeling and homeostasis; however, excessive activation in pathological states can lead to collagen degradation and inflammation. MMPs have a conserved structure, comprising a pro-peptide region for inactivity, a zinc-binding catalytic domain, and often a hemopexin-like domain that contributes to substrate specificity. Their activity requires precise regulation for proper ECM turnover and tissue repair, and dysregulation can lead to disease progression (9 – 12). MMPs are classified into different subgroups, such as collagenases and gelatinases, based on their functions and tissue localization. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are crucial enzymes that are directly involved in the degradation of type IV collagen and gelatin within the ECM. These gelatinases are essential for effective ECM remodeling, as they cleave basement membrane components. This action decisively promotes inflammatory cell infiltration and drives tissue turnover (9 – 12).

The pathophysiological activity of MMPs in AP is driven by their heightened expression and activation, which are decisively triggered by inflammatory mediators, immune cell signals, and ECM alterations (10 – 12). Extensive research in humans has demonstrated the critical role of MMPs in the breakdown of periapical tissue, intensifying the inflammatory response and facilitating the progression of the lesion (13). Considering the complex osteoimmunological interactions involved in alveolar bone resorption, the relationship between MMPs and bone resorption modulators [receptor activator of nuclear factor kappa-B (RANK), its ligand (RANKL), and osteoprotegerin (OPG)] remains poorly elucidated (14). It is a well-known fact that alveolar bone resorption is governed by the RANK/RANKL/OPG axis and other mediators, which tightly regulate osteoclast differentiation, activation, and activity in response to inflammatory

Uvod

Apikalni parodontitis (AP) kronična je imunosno-upalna bolest koja izravno utječe na periapikalna tkiva zahvaćenog zuba (1). Nastaje kao odgovor domaćina na polimikrobnu invaziju zubne pulpe. To stanje u osnovi je prouzročeno bakterijskom kolonizacijom unutar sustava korijenskog kanala, što uključuje stvaranje biofilmova i oslobađanje čimbenika virulencije. Ti čimbenici, zajedno s genetskom predispozicijom i drugim predisponirajućim faktorima, odlučno pokreću niz imunosnih odgovora u domaćinu (2 – 6). Zato je patogeneza AP-a potaknuta složenom i višestrukom mrežom koja integrira aktivaciju imunosnog sustava, regrutiranje različitih imunosnih stanica (npr., fagocita i limfocita) i proizvodnju različitih upalnih medijatora (npr., citokina, kemokina, reaktivnih vrsta kisika, matričnih metaloproteinaza – MMP-a, regulatora resorpcije kostiju itd) (7). Ti molekularni medijatori ključni su u usklađivanju degradacije izvanstaničnoga matriksa i resorpcije alveolarne kosti, što su glavne značajke u progresiji lezija (8).

Matriksne metaloproteinaze (MMP) cinkom ovisne su endopeptidaze koje razgrađuju kolagen i druge komponente izvanstanične matrice (ISM) (9 – 12). Njihovu aktivnost reguliraju tkivni inhibitori metaloproteinaza (TIMP) kako bi se spriječilo oštećenje tkiva. U normalnim uvjetima MMP-i olakšavaju preoblikovanje tkiva i homeostazu; no prekomjerna aktivacija u patološkim stanjima može rezultirati razgradnjom kolagena i upalom. MMP-i imaju konzerviranu strukturu koja se sastoji od propeptidne regije za neaktivnost, katalitičke domene koja veže cink i često domene slične hemopeksinu koji pridonosi specifičnosti supstrata. Njihova aktivnost zahtijeva preciznu regulaciju za pravilnu izmjenu ISM-a i popravak tkiva, a disregulacija može potaknuti progresiju bolesti (9 – 12). MMP-i se klasificiraju u različite podskupine poput kolagenaza i želatinaza na temelju njihovih funkcija i lokalizacije u tkivu. MMP-2 (želatinaza A) i MMP-9 (želatinaza B) ključni su enzimi koji su izravno uključeni u razgradnju kolagena tip IV i želatine unutar IMS-a. Te želatinaze važne su za učinkovito preoblikovanje ISM-a, jer cijepaju komponente bazalne membrane. To djelovanje odlučno potiče infiltraciju upalnih stanica i potiče obnovu tkiva (9 – 12).

Patofiziološka aktivnost MMP-a u AP-u prouzročena je njihovom pojačanom ekspresijom i aktivacijom koje odlučno pokreću upalni medijatori, signali imunosnih stanica i promjene ISM-a (10 – 12). Opsežna istraživanja na ljudima pokazala su ključnu ulogu MMP-a u razgradnji periapikalnog tkiva, intenziviranju upalnog odgovora i olakšavanju napredovanja lezije (13). S obzirom na složene osteoimunosne interakcije uključene u resorpciju alveolarne kosti, odnos između MMP-a i modulatora resorpcije kosti [receptor aktivator nuklearnog faktora kappa-β – RANK, njegov ligand – RANKL i osteoprotegerin – OPG) i dalje je slabo razjašnjen (14). Dobro je poznato da resorpciju alveolarne kosti reguliraju os RANK/RANKL/OPG i drugi medijatori koji strogo reguliraju diferencijaciju, aktivaciju i aktivnost osteoklasta kao odgovor na upalne i mikrobne znakove (15). Međutim, samo je u jednoj dosadašnjoj studiji, ali s malim uzorkom, ispitivan

and microbial cues (15). However, only one previous study with a small sample size examined the relationship between the presence of microbiota in infected root canals and the expression of mediators involved in the destruction of soft and bone tissue in apical periodontitis (16).

Therefore, this study aimed (a) to quantify the relative gene expression of *MMP-2, MMP-9, TIMP-2, RANKL*, and *OPG* in human AP and to associate it with levels of these molecules in healthy dental pulps; (b) to assess differences in gene expression among AP lesions stratified by clinical, radiographic, and histopathological features; (c) finally, the potential correlation of the investigated molecules and levels of MMPs in relations to the RANKL/OPG ratio were explored in AP samples.

Materials and Methods

Study design and cohorts of the investigation

This observational, cross-sectional clinical laboratory study was conducted at the Clinics of Oral Surgery and Restorative Odontology and Endodontics, as well as the Scientific Laboratories at the Implant Research Center, School of Dental Medicine, University of Belgrade, Serbia. This research protocol received approval from the Ethics Committee of the School of Dental Medicine, University of Belgrade (approval No. 36/11-2022). Before participation and any procedures, each patient or parents/legal guardians provided written informed consent. The investigation was carried out in strict compliance with established ethical standards, and in line with the principles outlined in the Helsinki Declaration of 1975, as revised in 2002.

The experimental cohort of the study comprised eighty consecutive patients recruited at the Clinics of Restorative Odontology and Oral Surgery, School of Dental Medicine, University of Belgrade, who sought dental care and were indicated for an apicoectomy procedure. In all cases, after thorough assessment of clinical and radiographic signs and symptoms of disease, surgical endodontic treatment was recommended due to the failure of standard root canal therapy. AP lesions were diagnosed using clinical (17) and radiographic examination [i.e., Periapical index (PAI) (18)]. Fifty consecutive patients who were scheduled for surgical extraction of impacted teeth or teeth needing removal due to ongoing orthodontic treatment at the Clinic of Oral Surgery, School of Dental Medicine, University of Belgrade, were included in the control group. Eligibility criteria for recruiting study cohorts are listed in Table 1.

The sample size calculation for this investigation was conducted using G*Power software (version 3.1, University of Düsseldorf, Germany), utilizing data from a previous study that analyzed the differences in relative gene expression of selected inflammatory mediators between patients with AP and healthy control samples (19). This calculation was further validated by testing a pilot sample from the current investigation. The analyses indicated that for a study comprising two groups, with a statistical power of 80% and a significance level of $\alpha = 0.05$, a minimum of 100 patients (50 in each group) was necessary.

odnos između mikrobiote u zaraženim korijenskim kanalima i ekspresije medijatora uključenih u uništavanje mekoga i koštanoga tkiva u apikalnom parodontitisu (16).

Zato su ciljevi ove studije bili:

a) kvantificirati relativnu ekspresiju gena *MMP-2, MMP-9, TIMP-2, RANKL* i *OPG* u ljudskome AP-u i povezati je s razinama tih molekula u zdravoj zubnoj pulpi
b) procijeniti razlike u ekspresiji gena među AP lezijama stratificiranima prema kliničkim, radiografskim i histopatološkim značajkama
c) istražiti potencijalnu korelaciju ispitivanih molekula i razina MMP-a u odnosu na omjer RANKL/OPG-a u uzorcima AP-a.

Materijali i metode

Dizajn studije i kohorte istraživanja

Ova opservacijska, presječna kliničko-laboratorijska studija provedena je na klinikama za oralnu kirurgiju i restaurativnu odontologiju i endodonciju te u znanstvenim laboratorijima Implantološko-istraživačkog centra Stomatološkog fakulteta Sveučilišta u Beogradu (Srbija). Ovaj istraživački protokol odobrilo je Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Beogradu (br. odobrenja 36/11-2022). Prije sudjelovanja i bilo kakvih postupaka, svaki pacijent ili roditelj/zakonski skrbnik potpisao je informirani pristanak. Istraživanje je provedeno strogo u skladu s etičkim standardima i načelima navedenima u Helsinškoj deklaraciji iz 1975., dopunjenoj 2002. godine.

Eksperimentalna skupina studije obuhvaća osamdeset pacijenata liječenih na Klinikama za restaurativnu odontologiju i oralnu kirurgiju Stomatološkog fakulteta Sveučilišta u Beogradu koji su zatražili stomatološku pomoć i indiciran im je postupak apikotomije. U svim slučajevima, poslije temeljite procjene kliničkih i radiografskih znakova i simptoma bolesti, preporučeno je kirurško endodontsko liječenje zbog neuspjeha standardne terapije korijenskog kanala. AP lezije dijagnosticirane su kliničkim (17) i radiografskim pregledom (tj. periapikalnim indeksom – PAI) (18). Pedeset uzastopnih pacijenata koji su bili određeni za kirurško vađenje impaktiranih zuba ili zuba koje je trebalo ukloniti zbog trenutačnog ortodontskog liječenja u Klinici za oralnu kirurgiju Stomatološkog fakulteta Sveučilišta u Beogradu, uključeni su u kontrolnu skupinu. Kriteriji za uključivanje u studijske skupine navedeni su u tablici 1.

Izračun veličine uzorka za ovo istraživanje proveden je u softveru G*Power (verzija 3.1, Sveučilište u Düsseldorfu, Njemačka), a koristili smo se podatcima iz prethodne studije u kojoj je analizirana razlika u relativnoj ekspresiji gena odabranih upalnih medijatora između pacijenata s AP-om i zdravih kontrolnih uzoraka (19). Taj izračun dodatno je potvrđen testiranjem pilot-uzorka iz trenutačnog istraživanja. Analize su pokazale da je za studiju koja obuhvaća dvije skupine, sa statističkom snagom od 80 % i razinom značajnosti $\alpha = 0,05$, potrebno najmanje 100 pacijenata (50 u svakoj skupini).

Table 1 Eligibility criteria for recruiting study cohorts
Tablica 1. Kriteriji za uključivanje u studijske kohorte

Inclusion criteria • Kriteriji uključivanja	Exclusion criteria*** • Kriteriji isključenja***
Indicated surgical endodontic treatment* • Indicirano kirurško endodontsko liječenje*	Periodontally involved teeth (probing depth >4 mm with periodontal bone loss) • Parodontalno zahvaćeni zubi (dubina sondiranja > 4 mm s gubitkom parodontalne kosti)
Indicated tooth removal due to impaction or orthodontic treatment** • Indicirano vađenje zuba zbog impakcije ili ortodontskog liječenja**	Vertical root fracture • Vertikalna fraktura korijena
Good general health (American Society of Anesthesia level 1 or 2)*** (20) • Dobro opće zdravstveno stanje (Američko društvo za anesteziju razinu 1 ili 2)*** (20)	Immunocompromised patients • Imunokompromitirani pacijenti
	Patients treated with antibiotics, antiviral, or immunosuppressive therapy 3 months before the procedures • Pacijenti liječeni antibioticima, antivirusnim lijekovima ili imunosupresivnom terapijom 3 mjeseca prije zahvata

* Strictly related to the patients in the experimental group; ** Strictly related to the patients in the control group; *** Related to the patients in both groups; * Strogo povezano s pacijentima u eksperimentalnoj skupini; ** Strogo povezano s pacijentima u kontrolnoj skupini; *** Povezano s pacijentima u objema skupinama

Table 2 Primer sequences and PCR conditions
Tablica 2 Sekvencije primera i uvjeti PCR-a

Gene • Gen	Primer sequence (5'→3') • Sekvencije primera (5'→3') Sense • Prednji Antisense • Povratni	Product size (bp) • Veličina produkta (bp)	PCR conditions • Uvjeti PCR-a
MMP2	5'-CTCCTGAATGCCCTTGATGT-3' 5'-ATGACAGCTGCACCACTGAG-3'	425	95 °C – 10 min, 45 cycles • krugova: · 95 °C – 30 s, · 55 °C – 30 s, · 72 °C – 30s, 72 °C – 10 min
MMP9	5'-GAACAAATACAGCTGGTTCC-3' 5'-TACCCTATGTACCGCTTCAC-3'	345	
TIMP2	5'-CTCTGTGACCCAGTCCATCC-3' 5'-ATGCACATCACCCTCTGTGA-3'	177	
RANKL	5'-CAGAAGATGGCACTCACTGCA-3' 5'-CACCATCGCTTTCTCTGCTCT-3'	203	
OPG	5'-GGAACCCCAGAGCGAAATACA-3' 5'-CCTGAAGAATGCCTCCTCACA-3'	225	
GAPDH	5'-TCATGACCACAGTCCATGCCATCA-3' 5'-CCCTGTTGCTGTAGCCAAATTCGT-3'	450	

Sampling, RNA extraction and Target Gene Expression assessment by Real-time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

One hundred thirty samples of the investigated tissues (80 apical periodontitis lesions and 50 healthy dental pulps) were collected following standard surgical procedures at the School of Dental Medicine, University of Belgrade, as previously described (18, 19). After harvesting, one portion of the AP lesion was used for histopathological examination and stored in a 10% formalin solution. Standard histopathological analysis of hematoxylin-eosin–stained slides was used for the stratification of periapical granulomas and radicular cysts (18, 19). Additionally, established classification systems were employed to further categorize AP lesions based on size and clinical phenotype (16 – 19). A second fragment of the AP lesion and collected healthy dental pulps were stored in RNAlater stabilization solution (Thermo Fisher Scientific, USA), refrigerated overnight, and kept at -70°C until RNA extraction and gene expression analysis.

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, USA), and cDNA was synthesized from 1 µg of RNA using the NG dART RT kit (EURx, Poland).

Uzorkovanje, ekstrakcija RNK i procjena ekspresije ciljnih gena s pomoću lančane reakcije polimeraze s reverznom transkripcijom u stvarnom vremenu (qRT-PCR)

Prema proceduri koja je ranije opisana, 130 uzoraka ispitivanih tkiva (80 AP lezija i 50 zdravih zubnih pulpi) prikupljeno je standardnim kirurškim postupcima na Stomatološkom fakultetu Sveučilišta u Beogradu (18, 19). Nakon skupljanja jedan dio AP lezije korišten je za histopatološki pregled te je bio pohranjen u 10-postotnoj otopini formalina. Standardna histopatološka analiza preparata obojenih hematoksilin-eozinom korištena je za stratifikaciju periapikalnih granuloma i radikularnih cista (18, 19). Uz to, korišteni su utvrđeni klasifikacijski sustavi za daljnju kategorizaciju AP lezija na temelju veličine i kliničkoga fenotipa (16 – 19). Drugi fragment AP lezije i prikupljene zdrave zubne pulpe pohranjeni su u otopini za stabilizaciju RNAlater (Thermo Fisher Scientific, SAD), hlađeni preko noći i držani na temperaturi od -70 °C do ekstrakcije RNK i analize ekspresije gena.

Ukupni RNK ekstrahiran je s pomoću reagensa TRIzol (Thermo Fisher Scientific, SAD), a cDNK sintetiziran je iz 1 µg RNK s pomoću kita NG dART RT (EURx, Poljska).

Gene expression levels of *RANKL, OPG, MMP2, MMP9*, and *TIMP2* were determined by SYBR Green-based qPCR using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA) with GAPDH used as the endogenous control. Reactions were performed in duplicate and specificity was confirmed by a melting curve analysis.

The cycling conditions and primer sequences are shown in Table 2. Relative expression was calculated using the 2^-ΔΔCt method, as described previously (19), based on the mean Ct value of duplicate reactions.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Numerical variables were described using means and standard deviations (SD). The normality of distribution for all values was assessed using the Kolmogorov–Smirnov test. As none of the gene expression variables followed a normal distribution, all between-group comparisons (e.g., case vs. control; different phenotypic subgroups) were conducted using non-parametric tests, specifically the Mann–Whitney U test (also known as the Wilcoxon rank-sum test). On the other hand, data which were related to age and sex were normally distributed and analyzed using the Chi-square test and Student's t-test. Correlations between gene expression levels and clinical parameters within the experimental group were analyzed using the Spearman's rank correlation coefficient. A significance level of $p < 0.05$ was considered statistically significant throughout.

Results

The experimental group included 34 men and 46 women, while the control group consisted of 22 men and 28 women (Chi-square test, p = 0.890). Patients in the experimental group were older than the ones in the control group (Student's t-test, 37.55±14.10 vs. 22.29±3.97, p < 0.001).

Higher gene expression levels of *RANKL* (p = 0.048), *OPG* (p = 0.002), *MMP2* (p = 0.048), and *MMP9* (p = 0.012) were observed in the experimental group compared to the control group. On the other hand, *TIMP2* expression was higher in the control group, however, this difference was not statistically significant (p = 0.120) (Table 3).

Phenotypically, of the total number of patients, 38 presented with symptomatic lesions, whereas 42 showed no symptoms. A total of 40 lesions were classified as large and 40 as small. As for the histopathological examination, 42 periapical granulomas and 38 radicular cysts were identified (Table 4).

Regarding the relative gene expression analysis, none of the markers showed differential expression associated with the presence or absence of symptoms (Table 4).

Higher relative expression of the *MMP2* gene was observed in smaller lesions (mean ± standard deviation (SD) 0.041 ± 0.088) compared to large lesions (mean ± SD 0.008 ± 0.015) (Mann-Whitney U-test p = 0.021, Table 4).

A similar trend was observed for granulomas compared to radicular cysts, although this comparison did not reach

Razine ekspresije gena *RANKL, OPG, MMP-2, MMP-9* i *TIMP-2* određene su SYBR Greenom baziranim na qPCR-u korištenjem SsoAdvanced™ Universal SYBR® Green Supermixa (Bio-Rad, SAD) s *GAPDH-om* kao endogenom kontrolom. Reakcije su provedene u duplikatu, a specifičnost je potvrđena analizom krivulje taljenja.

Uvjeti ciklusa i sekvencije primera prikazani su u tablici 2. Relativna ekspresija izračunata je metodom 2^-ΔΔCt, kako je već opisano (19), na temelju srednje Ct vrijednosti udvostručenih reakcija.

Statistička analiza

Sve statističke analize obavljene su u softveru GraphPad Prisma verzija 9.0 (GraphPad Software, San Diego, CA, SAD) i IBM SPSS Statistics verzija 26.0 (IBM Corp., Armonk, NY, SAD). Numeričke varijable opisane su korištenjem srednjih vrijednosti i standardnih devijacija (SD). Normalnost distribucije za sve vrijednosti procijenjena je Kolmogorov-Smirnovljevim testom. Budući da ni jedna od varijabli ekspresije gena nije slijedila normalnu distribuciju, sve usporedbe između skupina (npr., slučaj spram kontrole, različite fenotipske podskupine) provedene su korištenjem neparametrijskih testova, posebno Mann-Whitneyjeva U testa (poznatog i kao Wilcoxonov test zbroja rangova). S druge strane, podatci o dobi i spolu bili su normalno distribuirani i analizirani hi-kvadrat testom i Studentovim t-testom. Korelacije između razina ekspresije gena i kliničkih parametara unutar eksperimentalne skupine analizirane su korištenjem Spearmanova koeficijenta korelacije rangova. Razina značajnosti od p < 0,05 smatrana je statistički značajnom tijekom cijelog istraživanja.

Rezultati

Eksperimentalna skupina obuhvaćala je 34 muškarca i 46 žena, a kontrolna se sastojala od 22 muškarca i 28 žena (hi-kvadrat test, p = 0,890). Pacijenti u eksperimentalnoj skupini bili su stariji od onih u kontrolnoj (Studentov t-test, 37,55 ± 14,10 prema 22,29 ± 3,97, p < 0,001).

U eksperimentalnoj skupini, u usporedbi s kontrolnom, uočene su više razine ekspresije gena *RANKL* (p = 0,048), *OPG* (p = 0,002), *MMP-2* (p = 0,048) i *MMP-9* (p = 0,012). S druge strane, ekspresija gena *TIMP-2* bila je veća u kontrolnoj skupini, ali ta razlika nije bila statistički značajna (p = 0,120) (tablica 3.).

Fenotipski, od ukupnog broja pacijenata, 38 je imalo simptomatske lezije, a 42 nisu pokazivala znakove bolesti. Ukupno je 40 lezija klasificirano kao velike, a 40 kao male. Kad je riječ o histopatološkom pregledu, identificirana su 42 periapikalna granuloma i 38 radikularnih cista (tablica 4.).

Što se tiče analize relativne ekspresije gena, ni jedan od biljega nije pokazao diferencijalnu ekspresiju povezanu s prisutnošću ili odsutnošću simptoma (tablica 4.).

Veća relativna ekspresija gena *MMP-2* zabilježena je u manjim lezijama (srednja vrijednost ± standardna devijacija (SD) 0,041 ± 0,088) u usporedbi s velikim lezijama (srednja vrijednost ± SD 0,008 ± 0,015) (Mann-Whitneyjev U-test p = 0,021, tablica 4.).

Sličan trend uočen je kod granuloma u usporedbi s radikularnim cistama, iako ta usporedba nije dosegnula stati-

Table 3 The relative gene expression of *RANKL*, *OPG*, *MMP2*, *MMP9,* and *TIMP2* in the experimental and control groups.
Tablica 3. Relativna ekspresija gena *RANKL, OPG, MMP-2, MMP-9* i *TIMP-2* u eksperimentalnoj i kontrolnoj skupini

	RANKL	OPG	MMP2	MMP9	TIMP2
Experimental group (n=80) • Eksperimentalna skupina (n = 80)	0.006 ± 0.016	0.047 ± 0.194	0.024 ± 0.065	0.026 ± 0.155	0.305 ± 0.381
Control group (n=50) • Kontrolna skupina (n = 50)	0.004 ± 0.013	0.001 ± 0.002	0.008 ± 0.015	0.001 ± 0.002	0.720 ± 1.853
P value • *P* vrijednost	**0.048**	**0.002**	**0.048**	**0.012**	0.120

n-number of patients-tissue samples per group; RANKL-Receptor Activator of Nuclear factor Kappa-B Ligand; OPG-Osteoprotegerin; MMP-Matrix Metalloproteinase; TIMP-Tissue Inhibitor of matrix metalloproteinase; Values are presented as mean±standard deviations; Bold text denotes significant values • n – broj pacijenata – uzorci tkiva po skupini; RANKL – aktivator receptora nuklearnog faktora Kappa-B ligand; OPG – osteoprotegerin; MMP – matriksna metaloproteinaza; TIMP – tkivni inhibitor matriksne metaloproteinaze; vrijednosti su prikazane kao srednja vrijednost ± standardne devijacije; podebljani tekst označava značajne vrijednosti

Table 4 The relative gene expression of *RANKL*, *OPG*, *MMP2*, *MMP9,* and *TIMP2* in various phenotypic subgroups of apical periodontitis
Tablica 4. Relativna ekspresija gena *RANKL, OPG, MMP-2, MMP-9* i *TIMP-2* u različitim fenotipskim podskupinama apikalnog parodontitisa

	RANKL	OPG	MMP2	MMP9	TIMP2
Symptomatic lesion (n=38) • Simptomatske lezije (n = 38)	0.006 ± 0.020	0.064 ± 0.230	0.021 ± 0.055	0.017 ± 0.054	0.326 ± 0.435
Asymptomatic lesion (n=42) • Asimptomatske lezije (n = 4 2)	0.005 ± 0.010	0.031 ± 0.157	0.028 ± 0.073	0.035 ± 0.209	0.286 ± 0.328
P value • *P* vrijednost	0.718	0.977	0.458	0.908	0.707
Large AP lesion (n=40) • Velike lezije (n = 40)	0.006 ± 0.020	0.067 ± 0.225	0.008 ± 0.015	0.008 ± 0.019	0.273 ± 0.404
Small AP lesion (n=40) • Male lezije (n = 40)	0.006 ± 0.010	0.026 ± 0.158	0.041 ± 0.088	0.045 ± 0.219	0.336 ± 0.358
P value • *P* vrijednost	0.519	0.932	**0.021**	0.743	0.121
Periapical granulomas (n=42) • Periapiklani granulom (n = 42)	0.005 ± 0.010	0.025 ± 0.154	0.039 ± 0.086	0.043 ± 0.213	0.321 ± 0.356
Radicular cysts (n=38) • Radikularna cista (n = 38)	0.006 ± 0.020	0.071 ± 0.231	0.009 ± 0.015	0.008 ± 0.019	0.287 ± 0.410
P value • *P* vrijednost	0.441	0.776	0.062	0.862	0.214

n-number of patients-tissue samples per group; RANKL-Receptor Activator of Nuclear factor Kappa-B Ligand; OPG-Osteoprotegerin; MMP-Matrix Metalloproteinase; TIMP-Tissue Inhibitor of matrix metalloproteinase; Values are presented as mean±standard deviations; Bold text denotes significant values • n – broj pacijenata – uzorci tkiva po skupini; RANKL – aktivator receptora nuklearnog faktora Kappa-B ligand; OPG – osteoprotegerin; MMP – matriksna metaloproteinaza; TIMP – tkivni inhibitor matriksne metaloproteinaze; vrijednosti su prikazane kao srednja vrijednost ± standardne devijacije; podebljani tekst označava značajne vrijednosti.

statistical significance (Mann-Whitney U-test, p = 0.062, Table 4).

Higher relative expression of the *MMP2* gene was observed in lesions with *OPG* predominance (mean ± SD 0.039 ± 0.089) compared to lesions with *RANKL* predominance (mean ± SD 0.039 ± 0.089, Mann-Whitney U-test p = 0.021). Additionally, higher *TIMP2* expression was also found in lesions with *OPG* predominance (mean ± SD 0.017 ± 0.047) in comparison with lesions with *RANKL* predominance (mean ± SD 0.017 ± 0.047, Mann-Whitney U-test p = 0.021) (Figure 1).

Significant positive correlations were observed between the expression levels of bone resorption regulators and genes involved in extracellular matrix remodeling (*MMP2, MMP9*, and *TIMP2*) in the AP group (Figure 2).

Discussion

Alveolar bone resorption in AP is a dynamic process characterized by an intertwined network of inflammatory mediators (5). Previous research on humans has reported that MMPs have a significant impact on this process (18-28), but their relationship with bone resorption regulators remains poorly understood (13). This investigation clearly

stičku značajnost (Mann-Whitneyjev U-test, p = 0,062, tablica 4.).

Veća relativna ekspresija gena *MMP-2* uočena je u lezijama s predominacijom OPG-a (srednja vrijednost ± SD 0,039 ± 0,089) u usporedbi s lezijama s predominacijom RANKL-a (srednja vrijednost ± SD 0,039 ± 0,089, Mann-Whitneyjev U-test p = 0,021). Uz to, veća ekspresija gena *TIMP-2* pronađena je i u lezijama s predominacijom OPG-a (srednja vrijednost ± SD 0,017 ± 0,047) u usporedbi s lezijama s predominacijom RANKL-a (srednja vrijednost ± SD 0,017 ± 0,047, Mann-Whitneyjev U-test p = 0,021) (slika 1.).

Uočene su značajne pozitivne korelacije između razina ekspresije regulatora resorpcije kosti i gena uključenih u preoblikovanje izvanstaničnoga matriksa (*MMP-2, MMP-9* i *TIMP-2*) u AP skupini (slika 2.).

Rasprava

Resorpcija alveolarne kosti u AP-u dinamičan je proces obilježen isprepletenom mrežom upalnih medijatora (8). U dosadašnjim istraživanjima na ljudima autori su izvijestili da MMP-ovi znatno utječu na taj proces (21 – 31), ali njihov odnos s regulatorima resorpcije kosti ostaje slabo shvaćen (16). Ovo istraživanje jasno je pokazalo da su razine ekspresije ge-

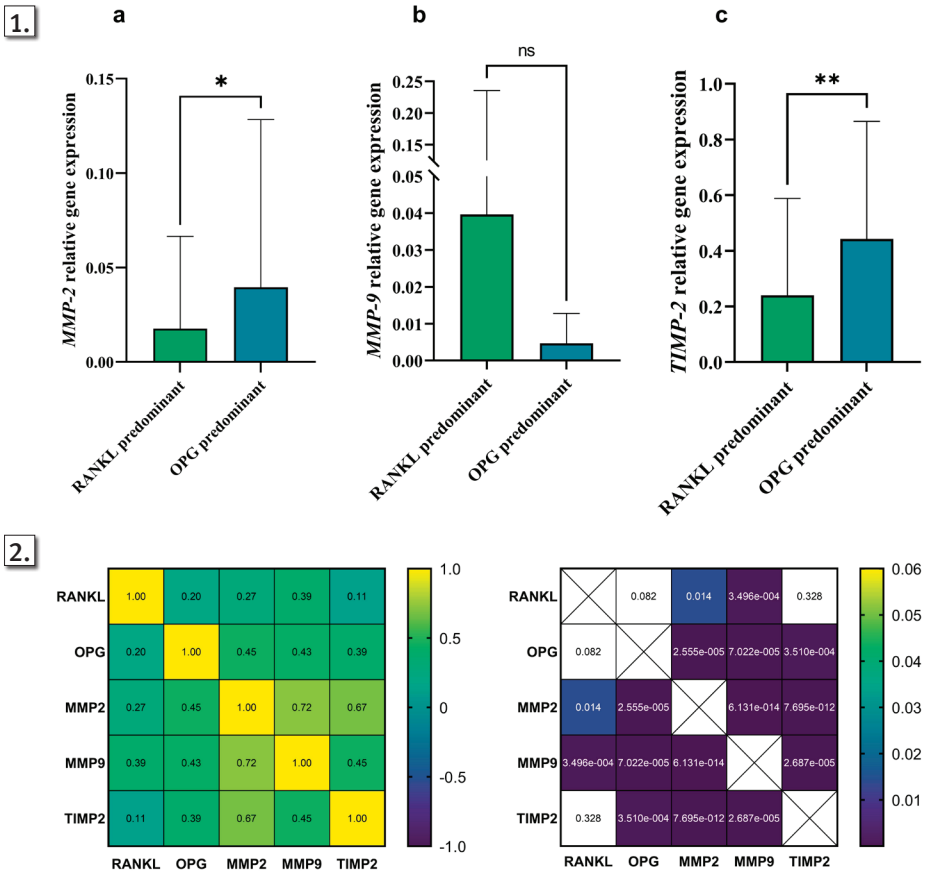

Figure 1 Expression patterns of selected matrix remodeling genes in RANKL- and OPG-predominant lesions. Panel (a) shows the relative gene expression levels of *MMP-2*, panel b of *MMP-9*, and panel c of *TIMP-2* in apical periodontitis lesions categorized based on RANKL or OPG predominance. Data are presented as mean ± SD, and statistical significance between groups was assessed using appropriate non-parametric tests. * and ** - statistically significant, ns - non-significant.

Slika 1. Obrasci ekspresije odabranih gena za preoblikovanje matrice u lezijama s predominantnim RANKL-ima i OPG-ima; panel a prikazuje relativne razine ekspresije gena *MMP-2*, panel b *MMP-9*, a panel c *TIMP-2* u lezijama apikalnog parodontitisa kategoriziranima na temelju predominantnosti RANKL-a ili OPG-a; podatci su prikazani kao srednja vrijednost ± SD, a statistička značajnost između skupina procijenjena je korištenjem odgovarajućih neparametrijskih testova. * i ** – statistički značajno, ns – neznačajno.

Figure 2 Spearman correlation heatmap and corresponding p-values. The left panel displays a heatmap of Spearman's rank correlation coefficients (ρ) among the analyzed variables. Lighter colors indicate stronger positive correlations. The right panel shows the corresponding p-values for each pairwise comparison, indicating the statistical significance of the observed correlations. The non-significant correlations ($p > 0.05$) are shown as white fields, with corresponding p values.

Slika 2. Spearmanova korelacijska toplinska karta i odgovarajuće p-vrijednosti; lijeva ploča prikazuje toplinsku kartu Spearmanovih koeficijenata korelacije ranga (ρ) među analiziranim varijablama; svjetlije boje označavaju jače pozitivne korelacije; desna ploča prikazuje odgovarajuće p-vrijednosti za svaku parnu usporedbu, što upućuje na statističku značajnost uočenih korelacija; neznačajne korelacije ($p > 0,05$) prikazane su kao bijela polja s odgovarajućim p-vrijednostima.

demonstrated that gene expression levels of *MMP-2*, *MMP-9*, *RANKL*, and *OPG* are significantly increased in human AP lesions compared to healthy dental pulps. These findings are supported by previous investigations confirming the role of inflammatory mediators in alveolar bone resorption (21 – 31). Shin et al (22), using enzyme-linked immunosorbent assay, pointed to increased levels of the investigated MMPs in periapical tissues compared to healthy pulps. In 2008, Belmar et al (23), using gelatin zymography, also revealed increased concentrations of MMP-2 and MMP-9 in gingival cervical fluid from teeth with periapical lesions compared to controls. The same result was confirmed by immunohistochemistry in several investigations (24, 25, 28, 29). Finally, using the same methodology applied in our research (i.e., RTqPCR), Letra et al (26) and Hadziabdic et al (27) confirmed the same trend.

na *MMP-2, MMP-9, RANKL* i *OPG* značajno povećane u ljudskim AP lezijama u usporedbi sa zdravom zubnom pulpom. Ove nalaze podupiru dosadašnja istraživanja u kojima je potvrđena uloga upalnih medijatora u resorpciji alveolarne kosti (21 – 31). Shin i suradnici (22), koristeći se enzimskim imunosorbentnim testom, istaknuli su povećane razine ispitivanih MMP-ova u periapikalnim tkivima u usporedbi sa zdravom pulpom. Godine 2008. Belmar i suradnici (23), koristeći se želatinskom zimografijom, također su otkrili povećane koncentracije MMP-2 i MMP-9 u gingivalnoj cervikalnoj tekućini zuba s periapikalnim lezijama u usporedbi s kontrolnom skupinom. Taj rezultat potvrđen je imunohistokemijski u nekoliko istraživanja (24, 25, 28, 29). Konačno, koristeći se istom metodologijom primijenjenom u našem istraživanju (tj. RTqPCR), Letra i suradnici (26) i Hadžiabdić i suradnici (27) potvrdili su isti trend.

As anticipated, this research demonstrates positive correlations between MMPs and the regulators of bone resorption, thus highlighting their critical interrelationship. Although these specific correlations have not been investigated before, our findings reinforce the conclusions drawn in previous studies. Hadziabdic et al (27) observed a significant correlation between *MMP-2* and *TIMP-2* in the analyzed sample. Moreover, Pereira Faustino et al (28) showed a positive moderate correlation between MMP-2 and MMP-9 expression in AP lesions. The same trend has been reported by Martinho et al (29), who confirmed that MMPs and large bone destruction positively correlate in AP.

This investigation also studied the difference in analyzed parameters between AP lesions with different phenotypes. Although there were no significant differences in the analyzed mediators between symptomatic and asymptomatic lesions, we observed that the MMP-2 was significantly increased in small-sized compared to large-sized lesions. In addition, the same tendency was observed in the levels of the analyzed parameter between periapical granulomas and radicular cysts. Our findings align with a larger cohort study conducted in Turkey (31), which found no significant difference in MMP-9 levels between symptomatic and asymptomatic acute AP lesions. In contrast, several other studies have reported contrary results, indicating that patients with clinical symptoms tend to have elevated levels of the MMPs studied (24, 25, 28, 30). These differences can be attributed to diverse methodological approaches employed in the investigations. First, there are obvious differences in the number of included samples analyzed among published studies. Then, different criteria were used to assess clinical conditions of the included patients among the analyzed studies. Another important issue is the type of sample that was used for analysis. In both experimental and control groups, a variety of samples were used, including gingival cervical fluid, periapical exudate, fresh periapical tissue, paraffin-embedded blocks of AP lesions, healthy pulp, healthy gingiva, or healthy periodontal ligament. Finally, studies used different tests to evaluate the presence or level of investigating molecules, including immunohistochemistry, zymography, enzyme-linked immunosorbent assay, real-time reverse transcription polymerase chain reaction, etc. These differences may significantly impact the results obtained, hence their interpretation should be approached with caution and careful consideration.

Concerning the size and histopathological features of AP lesions, as well as the levels of the tested parameters in our study, it is important to note that previously reported data have also shown significant diversity, and our findings are partially consistent with them. Although Hadziabdic et al (27) found no difference in MMPs levels between periapical granulomas and radicular cysts, they reported an increased relative gene expression of tested parameters in larger compared to small-sized AP lesions. No differences in MMP-9-positive cells between periapical granulomas and radicular cysts were also reported by Álvares et al (30). On the other hand, some studies reported increased levels of tested MMPs in periapical granulomas compared to radicular cyst (21, 28). These findings are backed by results from earlier animal stud-

Kao što se i očekivalo, ovo istraživanje pokazuje pozitivne korelacije između MMP-a i regulatora resorpcije kostiju te ističe njihovu ključnu uzajamnu povezanost. Iako ove specifične korelacije prije nisu bile istraživane, naši nalazi potvrđuju zaključke iz dosadašnjih studija. Hadžiabdić i suradnici (27) uočili su značajnu korelaciju između gena *MMP-2* i *TIMP-2* u analiziranom uzorku. Štoviše, Pereira Faustino i suradnici (28) pokazali su umjerenu pozitivnu korelaciju između ekspresije *MMP-2* i *MMP-9* u AP lezijama. O takvom trendu izvijestili su Martinho i suradnici (29) koji su potvrdili da MMP-i i velika destrukcija kostiju pozitivno koreliraju u AP-u.

U ovom istraživanju također se proučavala razliku u analiziranim parametrima između AP lezija s različitim fenotipovima. Iako nije bilo značajnih razlika u analiziranim medijatorima između simptomatskih i asimptomatskih lezija, primijetili smo da je MMP-2 značajno povećan u malim lezijama u usporedbi s velikima. Takva tendencija uočena je u razinama analiziranog parametra između periapikalnih granuloma i radikularnih cista. Naši nalazi u skladu su s većom kohortnom studijom provedenom u Turskoj (31) u kojoj nije pronađena značajna razlika u razinama MMP-9 između simptomatskih i asimptomatskih akutnih AP lezija. Suprotno tomu, u nekoliko drugih studija autori su izvijestili o suprotnim rezultatima, što upućuje na to da pacijenti s kliničkim simptomima imaju uglavnom povišenu razinu proučavanih MMP-a (24, 25, 28, 30). Te razlike mogu se pripisati drukčijim metodološkim pristupima korištenima u istraživanjima. Prvo, postoje očite razlike u broju analiziranih uzoraka među objavljenim studijama. Zatim, u analiziranim studijama korišteni su različiti kriteriji za procjenu kliničkoga stanja uključenih pacijenata. Drugo važno pitanje jest vrsta uzorka koji je upotrijebljen za analizu. I u eksperimentalnim i u kontrolnim skupinama uzeti su različiti uzorci, uključujući gingivalnu cervikalnu tekućinu, periapikalni eksudat, svježe periapikalno tkivo, parafinom ugrađene blokove AP lezija, zdravu pulpu, zdravu gingivu ili zdravi parodontni ligament. Konačno, autori studija služili su se različitim testovima za procjenu prisutnosti ili razine ispitivanih molekula, uključujući imunohistokemiju, zimografiju, enzimski imunosorbentni test, lančanu reakciju polimeraze s reverznom transkripcijom u stvarnom vremenu itd. Te razlike mogu značajno utjecati na dobivene rezultate i zato njihovoj interpretaciji treba pristupiti s oprezom i pozorno ih razmotriti.

Kad je riječ o veličini i histopatoloških značajki AP lezija te o razini testiranih parametara u našoj studiji, važno je napomenuti da su ranije objavljeni podatci također pokazali značajnu raznovrsnost, a naši nalazi djelomično su u skladu s njima. Iako Hadžiabdić i suradnici (27) nisu pronašli razliku u razinama MMP-a između periapikalnih granuloma i radikularnih cista, izvijestili su o povećanoj relativnoj ekspresiji gena testiranih parametara u većim AP lezijama u usporedbi s malima. Álvares i suradnici (30) također nisu izvijestili o razlikama u MMP-9-pozitivnim stanicama između periapikalnih granuloma i radikularnih cista. S druge strane, autori nekih studija izvijestili su o povećanim razinama testiranih MMP-a u periapikalnim granulomima u usporedbi s radikularnom cistom (21, 28). Te nalaze potkrepljuju rezultati rani-

ies. Corotti et al (32) showed that during the experimental development of AP, levels of MMPs were significantly higher in the early phase compared to the late phase. In this regard, Márton and Kiss (14) reviewed protective and destructive regulatory pathways in apical periodontitis development. An acute phase of disease is probably influenced by complex networking between different inflammatory cells and mediators that stimulate an increased production of MMPs and active degradation of the ECM.

It is also worth discussing that our patients with apical periodontitis were significantly older compared to those in the control group without periapical pathology. Although this might be a potential cofounding factor in this study design, it should be stated that it is in line with the findings reported by Hamedi et al (33), who investigated the prevalence of root canal treatment and periapical radiolucency in elderly patients. The authors clearly demonstrated that in comparison to general adult populations, the elderly had a much higher prevalence of non-surgical root canal treatment and a higher prevalence of periapical radiolucency. Therefore, following the observed trend, our demographic results support the fact that the elderly population has an increased prevalence of apical periodontitis.

This investigation demonstrates several strengths, including a predetermined sample size, a large cohort of samples, and robust laboratory methodology. However, it is crucial to acknowledge certain limitations. The cross-sectional design in Endodontology restricts the ability to establish strong causality between the analyzed genes and the development of apical periodontitis due to ethical considerations. Furthermore, it is essential to match the ages of patients in the experimental group with those of patients who do not present periapical radiolucency, as potential confounding factors can impact the results.

Taken together, it has been demonstrated that the resorption of alveolar bone in AP is a complex process influenced by a variety of inflammatory mediators. The observed upregulation of *MMP-2, MMP-9, RANKL,* and *OPG* in AP lesions emphasizes their potential role as molecular biomarkers for disease activity and progression. These findings could enhance diagnostic accuracy, thus enabling better patient stratification and personalized treatment strategies in endodontic practice, as demonstrated in Periodontology (34, 35). Moreover, elucidating the interplay between MMPs and bone resorption regulators may open new avenues for targeted pharmacological interventions aimed at modulating periapical inflammation and preventing alveolar bone destruction. Future studies with larger cohorts, standardized sampling protocols, and longitudinal designs are warranted to validate these biomarkers, explore their predictive value for treatment outcomes, and assess their potential utility in personalized management of AP.

Conclusions

This study confirms the coordinated involvement of MMPs and bone resorption regulators in apical periodontitis lesions. Further research is required to validate their clinical

jih studija na životinjama. Corotti i suradnici (32) pokazali su da su tijekom eksperimentalnog razvoja AP razine MMP-a bile značajno više u ranoj fazi u usporedbi s kasnom fazom. U tom smislu, Márton i Kiss (14) pregledali su zaštitne i destruktivne regulatorne putove u razvoju apikalnog parodontitisa. Akutna faza bolesti vjerojatno je pod utjecajem složene mreže između različitih upalnih stanica i medijatora koji stimuliraju povećanu proizvodnju MMP-ova i aktivnu razgradnju izvanstaničnoga matičnog tkiva.

Također valja spomenuti da su naši pacijenti s apikalnim parodontitisom bili puno stariji u usporedbi s onima u kontrolnoj skupini bez periapikalne patologije. Iako bi to mogao biti suosnivački čimbenik u dizajnu ove studije, treba istaknuti da je to u skladu s nalazima o kojima su izvijestili Hamedi i suradnici (33) koji su istraživali prevalenciju liječenja korijenskog kanala i periapikalne radiolucencije kod starijih osoba. Autori su jasno pokazali da su u usporedbi s općom odraslom populacijom stariji imali mnogo veću prevalenciju nekirurškog liječenja korijenskog kanala i veću prevalenciju periapikalne radiolucencije. Zato, slijedeći uočeni trend, naši demografski rezultati podupiru činjenicu da starija populacija ima povećanu prevalenciju apikalnog parodontitisa.

Ovo istraživanje ima nekoliko prednosti, uključujući unaprijed određenu veličinu uzorka, veliku kohortu uzoraka i robusnu laboratorijsku metodologiju. No važno je priznati određena ograničenja. Presječni dizajn u endodontologiji ograničava mogućnost uspostavljanja snažne uzročnosti između analiziranih gena i razvoja apikalnog parodontitisa zbog etičkih razmatranja. Nadalje, bitno je uskladiti dob pacijenata u eksperimentalnoj skupini s dobnom skupinom pacijenata koji nemaju periapikalnu radiolucenciju, jer potencijalni zbunjujući čimbenici mogu utjecati na rezultate.

Uzevši sve u obzir, dokazano je da je resorpcija alveolarne kosti u AP-u složen proces na koji utječu razni upalni medijatori. Uočena povišena regulacija *MMP-2, MMP-9, RANKL-a* i *OPG-a* u AP lezijama ističe njihovu potencijalnu ulogu kao molekularnih bioloških biljega za aktivnost i progresiju bolesti. Ti nalazi mogli bi poboljšati dijagnostičku točnost i omogućiti bolju stratifikaciju pacijenata i personaliziranu strategiju liječenja u endodontskoj praksi, kao što je istaknuto u Parodontologiji (34, 35). Štoviše, razjašnjavanje međudjelovanja između MMP-ova i regulatora resorpcije kosti može otvoriti nove smjerove za ciljane farmakološke intervencije usmjerene na modulaciju periapikalne upale i sprječavanje uništavanja alveolarne kosti. Potrebne su nove studije s većim kohortama, standardiziranim protokolima uzorkovanja i longitudinalnim dizajnom da bi se validirali ti biološki biljezi, istražila njihova prediktivna vrijednost za ishode liječenja i procijenila potencijalna korisnost u personaliziranom liječenju AP-a.

Zaključak

Ova studija potvrđuje koordiniranu uključenost MMP-a i regulatora resorpcije kosti u periapikalnim lezijama. Potrebna su daljnja istraživanja kako bi se potvrdila njihova kli-

relevance and explore their potential role in improving diagnostic and therapeutic approaches.

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

Funding: Ministry of Science, Republic of Serbia, contract no. 451-03-137/2025-03/200129

Ethical approvals: The research protocol of this investigation was assessed and approved by the Ethical Committee of the School of Dental Medicine, University of Belgrade, Serbia (approval number: 36/11-2022)

Author's contribution: All authors participated equally in all phases of this project

nička relevantnost i istražila potencijalna uloga u poboljšanju dijagnostičkih i terapijskih pristupa.

Sukob interesa: Autori nisu bili u sukobu interesa.

Financiranje: Ministarstvo nauke Republike Srbije broj ugovora: 451-03-137/2025-03/200129

Etičko odobrenje: Istraživački protokol ovog istraživanja procijenilo je i odobrilo Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Beogradu (broj odobrenja: 36/11-2022).

Doprinos autora: Svi autori podjednako su sudjelovali u svim fazama projekta.

Sažetak

Svrha rada: Kvantificirati relativnu ekspresiju gena matričnih metaloproteinaza (MMP)-2, -9, njihov tkivni inhibitor (TIMP)-2, ligandu receptora aktivatora nuklearnog faktora kappa-β (*RANKL*) i osteoprotegerin (*OPG*) u uzorcima humanoga apikalnog parodontitisa (AP) te je povezati s razinama tih molekula u zdravoj zubnoj pulpi. Procijeniti razlike u ekspresiji gena među AP lezijama stratificiranima prema kliničkim, radiografskim i histopatološkim značajkama. I na kraju, u uzorcima AP-a istražiti potencijalnu korelaciju analiziranih molekula. **Materijali i metode:** Studijska kohorta sastojala se od 80 AP lezija dobivenih od 80 odraslih dobrovoljaca tijekom postupaka apikotomije. Zdrava kontrolna kohorta sastojala se od 50 uzoraka zubne pulpe s intaktnih zuba ekstrahiranih u ortodontske svrhe prikupljenih od 50 darivatelja. Relativna ekspresija gena *MMP-2, MMP-9, TIMP-2, RANKL* i *OPG* procijenjena je u svim uzorcima tkiva korištenjem kvantitativne lančane reakcije polimeraze u stvarnom vremenu s reverznom transkripcijom (RT-qPCR). Statističke analize uključivale su hi-kvadrat test, Studentov t-test, Mann-Whitneyjev U test i Spearmanovu korelaciju. **Rezultati:** U studiji su uočene više razine ekspresije gena *RANKL, OPG, MMP-2* i *MMP-9* u usporedbi sa zdravom kohortom ($p = 0,048$, $p = 0,002$, $p = 0,048$ i $p = 0,012$). Veća relativna ekspresija gena *MMP-2* zabilježena je u manjim lezijama u usporedbi s velikim lezijama ($p = 0,021$). Uočene su značajne pozitivne korelacije između ispitivanih medijatora. **Zaključci:** Istodobna prekomjerna ekspresija proučavanih medijatora, zajedno s njihovim pozitivnim korelacijama u AP lezijama, upućuje na njihovu koordiniranu ulogu u progresiji bolesti i resorpciji alveolarne kosti.

Zaprimljen: 28. kolovoza 2025.
Prihvaćen: 3. listopada 2025.

Adresa za dopisivanje
Aleksandar Jakovljević,
Odsjek za patološku fiziologiju,
Stomatološki fakultet, Sveučilište u Beogradu, Srbija,
Dr. Subotića 8, 11 000 Beograd, Srbija.
telefon: +381112685288;
a.jakovljevic@stomf.bg.ac.rs

MeSH pojmovi: periapikalni parodontitis; posrednici upale; matriksne metaloproteinaze; resorpcija kosti; gubitak alveolarne kosti
Autorske ključne riječi: apikalni parodontitis, medijatori upale, matriksne metaloproteinaze, resorpcija kosti

References

1. Wen YH, Lin YX, Zhou L, Lin C, Zhang L. The immune landscape in apical periodontitis: From mechanism to therapy. Int Endod J. 2024;57(11):1526-1545. doi: 10.1111/iej.14125.
2. Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol. 2011;33: 5304. doi: 10.3402/jom.v3i0.5304.
3. Pan D, Hao Y, Tao Y, Li B, Cheng L. The influence of microorganisms on bone homeostasis in apical periodontitis. Arch Oral Biol. 2025;170:106153. doi: 10.1016/j.archoralbio.2024.106153.
4. Kadić M, Nikolić N, Carkić J, Kadić-Pirović A, Beljić-Ivanović K, Andrić M i sur. Prevalence of Glutathione-S-Transferase T and M Deletion Polymorphisms in Apical Periodontitis: a Two-Center Observational Study. Acta stomatol Croat. 2025;59(3):226-235. https://doi.org/10.15644/asc59/3/1
5. de Oliveira BP, Cruz Câmara A, Menezes Aguiar C. Prevalence of Asymptomatic Apical Periodontitis and its Association with Coronary Artery Disease in a Brazilian Subpopulation. Acta stomatol Croat. 2017;51(2):106-112. https://doi.org/10.15644/asc51/2/3
6. Jakovljevic A, Jacimovic J, Georgiou AC, Nikolic N, Aminoshariae A, van der Waal SV, Nagendrababu V. Single nucleotide polymorphisms as a predisposing factor for the development of apical periodontitis-An umbrella review. Int Endod J. 2022;55(7):700-713. doi: 10.1111/iej.13756.
7. Luo X, Wan Q, Cheng L, Xu R. Mechanisms of bone remodeling and therapeutic strategies in chronic apical periodontitis. Front Cell Infect Microbiol. 2022;12:908859. doi: 10.3389/fcimb.2022.908859.
8. Milojevic N, Krdzovic Lazic E, Lukic L, Puresevic D, Mirkovic M, Jakovljevic A. Inflammatory mediators' essence in apical periodontitis. Eur Oral Res. 2024;58(3):160-168. doi: 10.26650/eor.20241423117.
9. Laronha H, Caldeira J. Structure and Function of Human Matrix Metalloproteinases. Cells. 2020;9(5):1076. doi: 10.3390/cells9051076.
10. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand. 2007;65(1):1-13. doi: 10.1080/00016350600963640.
11. Jain A, Bahuguna R. Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview. J Oral Biol Craniofac Res. 2015;5(3):212-8. doi: 10.1016/j.jobcr.2015.06.015.
12. Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The Significance of Matrix Metalloproteinases in Oral Diseases. Adv Clin Exp Med. 2016;25(2):383-90. doi: 10.17219/acem/30428.
13. Torres AFC, Antunes LS, Oliveira NF, Küchler EC, Gomes CC, Antunes LAA. Genetic Polymorphism and Expression of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Periapical Lesions: Systematic Review. J Endod. 2020;46(1):3-11.e1. doi: 10.1016/j.joen.2019.10.011.
14. Márton IJ, Kiss C. Overlapping protective and destructive regulatory pathways in apical periodontitis. J Endod. 2014;40(2):155-63. doi: 10.1016/j.joen.2013.10.036.
15. Belibasakis GN, Rechenberg DK, Zehnder M. The receptor activator of NF-κB ligand-osteoprotegerin system in pulpal and periapical disease. Int Endod J. 2013;46(2):99-111. doi: 10.1111/j.1365-2591.2012.02105.x.
16. Takahama A Jr, Rôças IN, Faustino ISP, Alves FRF, Azevedo RS, Gomes CC, Araújo-Filho WR, Siqueira JF Jr. Association between bacteria occurring in the apical canal system and expression of bone-resorbing mediators and matrix metalloproteinases in apical periodontitis. Int Endod J. 2018;51(7):738-746. doi: 10.1111/iej.12895.
17. Jakovljevic A, Andric M, Knezevic A, Soldatovic I, Nikolic N, Karalic D, Milasin J. Human Cytomegalovirus and Epstein-Barr Virus Genotypes in Apical Periodontitis Lesions. J Endod. 2015;41(11):1847-51. doi: 10.1016/j.joen.2015.08.027.
18. Orstavik D, Kerekes K, Eriksen HM. The periapical index: a scoring system for radiographic assessment of apical periodontitis.

Endod Dent Traumatol. 1986;2(1):20-34. doi: 10.1111/j.1600-9657.1986.tb00119.x.
19. Nikolic N, Jakovljevic A, Carkic J, Beljic-Ivanovic K, Miletic M, Soldatovic I, Andric M, Ivanovic V, Milasin J. Notch Signaling Pathway in Apical Periodontitis: Correlation with Bone Resorption Regulators and Proinflammatory Cytokines. J Endod. 2019;45(2):123-128. doi: 10.1016/j.joen.2018.10.015.
20. Wolters U, Wolf T, Stützer H, Schröder T. ASA classification and perioperative variables as predictors of postoperative outcome. Br J Anaesth. 1996;77(2):217-22. doi: 10.1093/bja/77.2.217.
21. de Paula-Silva FW, D'Silva NJ, da Silva LA, Kapila YL. High matrix metalloproteinase activity is a hallmark of periapical granulomas. J Endod. 2009;35(9):1234-42. doi: 10.1016/j.joen.2009.06.008.
22. Shin SJ, Lee JI, Baek SH, Lim SS. Tissue levels of matrix metalloproteinases in pulps and periapical lesions. J Endod. 2002;28(4):313-5. doi: 10.1097/00004770-200204000-00013.
23. Belmar MJ, Pabst C, Martínez B, Hernández M. Gelatinolytic activity in gingival crevicular fluid from teeth with periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(6):801-6. doi: 10.1016/j.tripleo.2007.12.002.
24. Carneiro E, Menezes R, Garlet GP, Garcia RB, Bramante CM, Figueira R, Sogayar M, Granjeiro JM. Expression analysis of matrix metalloproteinase-9 in epithelialized and nonepithelialized apical periodontitis lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(1):127-32. doi: 10.1016/j.tripleo.2008.07.030.
25. Ahmed GM, El-Baz AA, Hashem AA, Shalaan AK. Expression levels of matrix metalloproteinase-9 and gram-negative bacteria in symptomatic and asymptomatic periapical lesions. J Endod. 2013;39(4):444-8. doi: 10.1016/j.joen.2012.11.009.
26. Letra A, Ghaneh G, Zhao M, Ray H Jr, Francisconi CF, Garlet GP, Silva RM. MMP-7 and TIMP-1, new targets in predicting poor wound healing in apical periodontitis. J Endod. 2013;39(9):1141-6. doi: 10.1016/j.joen.2013.06.015.
27. Hadziabdic N, Kurtovic-Kozaric A, Pojskic N, Sulejmanagic N, Todorovic L. Gene-expression analysis of matrix metalloproteinases 1 and 2 and their tissue inhibitors in chronic periapical inflammatory lesions. J Oral Pathol Med. 2016;45(3):224-30. doi: 10.1111/jop.12347.
28. Pereira Faustino IS, Azevedo RS, Takahama A Jr. 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. 2016;42(4):547-51. doi: 10.1016/j.joen.2015.12.020.
29. Martinho FC, Teixeira FF, Cardoso FG, Ferreira NS, Nascimento GG, Carvalho CA, Valera MC. 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. 2016;42(7):1082-8. doi: 10.1016/j.joen.2016.04.001.
30. Álvares PR, Arruda JAA, Silva LPD, Nascimento GJFD, Silveira MFD, Sobral APV. Immunohistochemical expression of TGF-β1 and MMP-9 in periapical lesions. Braz Oral Res. 2017;31:e51. doi: 10.1590/1807-3107BOR-2017.vol31.0051.
31. Ozdemir B, Ersahan S, Ozcelik F, Hepsenoglu YE, Sirin DA, Topbas C. Relationship of MMP-9 with the clinical course of apical periodontitis and the main bacterial species in the oral microbiota. Odontology. 2024;112(3):976-987. doi: 10.1007/s10266-023-00895-w.
32. Corotti MV, Zambuzzi WF, Paiva KB, Menezes R, Pinto LC, Lara VS, Granjeiro JM. Immunolocalization of matrix metalloproteinases-2 and -9 during apical periodontitis development. Arch Oral Biol. 2009;54(8):764-71. doi: 10.1016/j.archoralbio.2009.04.013.
33. Hamedy R, Shakiba B, Pak JG, Barbizam JV, Ogawa RS, White SN. Prevalence of root canal treatment and periapical radiolucency in elders: a systematic review. Gerodontology. 2016;33(1):116-27. doi: 10.1111/ger.12137.
34. Franco C, Patricia HR, Timo S, Claudia B, Marcela H. Matrix Metalloproteinases as Regulators of Periodontal Inflammation. Int J Mol Sci. 2017 Feb 17;18(2):440. doi: 10.3390/ijms18020440.
35. Šumilin L, Musić L, Puhar I, Sabol I, Japirko I, Kuna K, Lončar BB. Diagnostic Accuracy of Salivary aMMP-8 Test in Infertile Women and Blood Finding Analysis. Acta Stomatol Croat. 2022;56(2):98-108. doi: 10.15644/asc56/2/1.