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Prevalence of Glutathione-S-Transferase T and M Deletion Polymorphisms in Apical Periodontitis: a Two-Center Observational Study

Prevalencija delecijskih polimorfizama za glutation S-transferazu T i M u apikalnom parodontitisu – dvocentrična opservacijska studija

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Abstract

Objectives: The primary objective of this study was to examine the potential association between glutathione S-transferases (*GSTT1/GSTM1*) deletion polymorphisms and the development of apical periodontitis (AP) in a population of patients at two university centers: the Faculty of Medicine at the University of Banja Luka in Bosnia and Herzegovina and the School of Dental Medicine at the University of Belgrade in Serbia. **Materials and Methods:** The study involved 200 patients with AP in the experimental and 250 healthy individuals without AP in the control group. As a source of genomic DNA, sterile buccal swabs were taken from each patient. Genotyping of *GSTM1* and *GSTT1* deletion polymorphisms was conducted using multiplex Polymerase Chain Reaction (PCR). The risk of AP development with regard to the genotypes was evaluated based on odds ratios (ORs) and 95% confidence intervals (CIs) that were calculated via unconditional logistic regression. **Results:** There were significant differences in demographic characteristics between the investigated groups (p = 0.446, p = 0.154, respectively). *GSTM1* and *GSTT1* deletions were associated with a 3.05-fold and 5.69-fold risk (OR = 3.05, 95% CI = 2.07–4.49, OR = 5.69, 95% CI = 3.66–8.86, p < 0.001, p < 0.001, respectively) for the AP development. The co-occurrence of both deletions posed a significantly higher risk for AP development (OR = 52.76. 95% CI = 18.20–152.94, P < 0.001). **Conclusions:** The carriers of null *GSTT*, null *GSTM*, and double null *GSTT/GSTM* genotypes are more susceptible to AP development in the populations examined at the two centers.

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Introduction

Apical periodontitis (AP) represents a local and systemic inflammatory response of the body to various stimuli that compromise the vitality of the dental pulp, ultimately leading to its necrosis (1). Over the last twenty years, this condition has been alarmingly prevalent worldwide, with a significant increase noted among both untreated and endodontically treated teeth (2, 3). When contextualizing the burden of AP within the broader spectrum of chronic oral conditions, it is important to compare it with marginal periodontitis, a highly prevalent and impactful disease. Recent meta-analysis reports that AP affects approximately 6.3% of teeth in the general adult population, with a prevalence of 41.3% among endodontically treated teeth (2). In contrast, marginal periodontitis has a substantially higher overall prevalence, estimated at 61.6% in dentate adults, with severe forms reaching 23.6% (4). These comparisons underscore the clinical and epidemiological significance of both endodontic and periodontal diseases in global oral health. The widespread occurrence of AP is closely linked to numerous general health impairments and imposes a considerable financial burden on healthcare systems around the globe (5-8). Nevertheless, root canal therapy followed by proper restoration is generally more cost-effective compared to extraction and subsequent prosthetic rehabilitation, such as implants or bridges, which are associated with higher upfront and long-term maintenance costs (9).

The pathogenesis of AP is a complex process characterized by overlapping signaling pathways and inflammatory mediators that drive progressive bone resorption in the periapical region of the affected tooth. Central to this alveolar bone resorption is a definitive bidirectional relationship between multiple etiological factors and the response of the host's immune system (10, 11). It is a well-established that polymicrobial infection affecting the dental pulp that represents the leading cause of apical periodontitis (AP) (12). Furthermore, various factors, including environmental and predisposing elements, have a critical role in inducing and exacerbating the progression of the disease (13).

Numerous investigations have highlighted the influence of heredity on the development of AP (14-17). Recent reviews suggest that specific genotypes of certain genes across various populations are linked to the occurrence and progression of AP, along with its diverse clinical and radiographic manifestations (14-17). However, no definitive genetic marker has been identified that can reliably predict a person's susceptibility to developing apical periodontitis.

Oxidative stress (OS) arises from a disproportion between the generation of reactive oxygen species (ROS) and the capacity of antioxidant systems to neutralize them, which subsequently leads to cellular damage and inflammation. Previous studies clearly demonstrated the significance of OS in the pathogenesis of apical periodontitis (18). Glutathione S-transferases (GSTs) represent a diverse superfamily of phase II metabolic enzymes that catalyze the conjugation of the tripeptide glutathione (GSH) to a vast array of endogenous and exogenous electrophilic substrates (19, 20). This conjugation pro-

Uvod

Apikalni parodontitis (AP) lokalni je i sistemski upalni odgovor tijela na različite podražaje koji ugrožavaju vitalnost zubne pulpe, što u konačnici rezultira njezinom nekrozom (1). To je stanje alarmantno rašireno diljem svijeta, sa značajnim porastom zabilježenim među neliječenim i endodontski liječenim zubima u posljednjih dvadeset godina (2, 3). Tijekom analize učestalosti AP-a unutar šireg spektra kroničnih oralnih stanja, važno ga je usporediti s marginalnim parodontitisom, vrlo raširenom i čestom bolešću. U nedavnoj metaanalizi ističe se da AP zahvaća približno 6,3 % zuba u općoj odrasloj populaciji, s prevalencijom od 41,3 % među endodontski liječenim zubima (2). Suprotno tomu, marginalni parodontitis ima znatno veću ukupnu prevalenciju procijenjenu na 61,6 % kod odraslih osoba, i s teškim oblicima koji dosižu 23,6 % učestalosti (4). Te usporedbe ističu kliničko i epidemiološko značenje i endodontskih i parodontalnih bolesti u globalnome oralnom zdravlju. Široko rasprostranjena pojava AP-a usko je povezana s mnogobrojnim općim zdravstvenim bolestima i nameće znatan financijski teret zdravstvenim sustavima diljem svijeta (5 – 8). Ipak, liječenje inficiranih kanala korijena zahvaćenih zuba, poslije čega slijedi pravilna restauracija, općenito je isplativije u usporedbi s vađenjem i naknadnom protetičkom rehabilitacijom, poput implantata ili mostova koji su povezani s većim početnim i dugoročnim troškovima održavanja (9).

Patogeneza AP-a složen je proces koji karakterizira preklapajuća uloga signalnih putova i upalnih medijatora koji potiču progresivnu resorpciju kosti u periapikalnom području zahvaćenog zuba. Središnji dio resorpcije alveolarne kosti definitivni je dvosmjerni odnos između više etioloških čimbenika i odgovora imunosnog sustava domaćina (10, 11). Dobro je poznato da je polimikrobna infekcija koja inficira zubnu pulpu glavni uzrok apikalnog parodontitisa (12). Nadalje, različiti čimbenici, uključujući okolišne i predisponirajuće elemente, ključni su u izazivanju i pogoršanju progresije bolesti (13).

Autori mnogobrojnih istraživanja istaknuli su utjecaj nasljednosti kad je riječ o pojavi AP-a (14 – 17). Nedavni pregledni radovi sugeriraju da su specifični genotipovi određenih gena u različitim populacijama povezani s pojavom i napredovanjem AP-a te da utječu na njegove različite kliničke i radiografske manifestacije (14 – 17). No još nije otkriven definitivni genetski biljeg kojim se može pouzdano predvidjeti sklonost osobe prema nastanku apikalnog parodontitisa.

Oksidacijski stres (OS) nastaje zbog nerazmjera između stvaranja reaktivnih kisikovih vrsta (ROS) i svojstva antioksidacijskih sustava da ih neutraliziraju, što rezultira oštećenjem stanica i upalama. U dosadašnjim studijama jasno je istaknuto značenje OS-a u patogenezi apikalnog parodontitisa (18). Glutation S-transferaze (GST) raznolika su superobitelj metaboličkih enzima faze II koji kataliziraju konjugaciju tripeptida glutationa (GSH) s velikim brojem endogenih i egzogenih elektrofilnih supstrata (19, 20). Taj proces konjugacije olakšava solubilizaciju, detoksikaciju i naknadno izlučivanje spojeva koji mogu biti štetni. Smanjivanjem na-

cess facilitates solubilization, detoxification, and subsequent excretion of the compounds that may be potentially harmful. By mitigating the accumulation of reactive intermediates, GSTs significantly contribute to redox homeostasis and protect cells from oxidative stress, lipid peroxidation, and DNA damage (19, 20). Among the cytosolic classes, GSTM1 (mu), GSTT1 (theta), and GSTP1 (pi) have garnered significant attention in dental research owing to their pronounced polymorphic variability and their implication in the pathophysiology of various inflammatory, neoplastic, and degenerative diseases (19, 20). It has been postulated that disruptions in GST function, due to genetic deletions (e.g., in carriers with *GSTM1* or *GSTT1* null genotypes), may compromise their protective capacity, thereby contributing to heightened tissue destruction and disease progression (19, 20).

A few studies have previously explored the connection between various *GST* polymorphisms and the presence of marginal periodontitis (21-26). Their findings highlighted the important role of genetic variations in GST enzymes, particularly *GSTM1, GSTT1*, and *GSTP1*, in influencing individual susceptibility to chronic periodontitis. Research has shown that people who have null genotypes of *GSTM1* and *GSTT1*, or those carrying the variant allele (G) at the rs1695 locus of *GSTP1*, exhibit compromised antioxidant defense, rendering them more susceptible to oxidative stress and inflammatory damage in periodontal tissues (21-26).

Although marginal and apical periodontitis shared similar pathological features, evidence regarding the impact of *GST* polymorphisms on AP development is lacking. Therefore, the primary objective of this study was to examine a potential association between *GSTT/GSTM* deletion polymorphisms and the development of apical periodontitis in a population of patients at two university centers: the Faculty of Medicine at the University of Banja Luka in Bosnia and Herzegovina and the School of Dental Medicine at the University of Belgrade in Serbia.

Materials and Methods

The present study represents clinical-laboratory and observational genetic association research. The investigation was performed and reported in alignment with the Strengthening the Reporting of Genetic Association study (STREGA) statement checklist (27) (Supplementary Table 1) and the Preferred Reporting Items for Laboratory and Observational Studies in Endodontology (PRILE, PROBE) guidelines (28, 29).

Ethical considerations

The protocol followed in this two-center investigation was reviewed and approved by both local institutional Research Ethics Committees (protocol numbers: 36/12-2013, and 02-3-4-19-1-6/2022). All study participants or parents/legal guardians provided informed written consent, i.e., an assent document, at both centers. Moreover, the procedures followed were all in accordance with the ethical standards of both the responsible committees on human experimentation (University of Banja Luka, B&H, and University of Belgrade, Serbia) and with the Helsinki Declaration of 1975, as revised in 2002.

kupljanja reaktivnih međuprodukata, GST-i značajno pridonose redoks homeostazi i štite stanice od oksidacijskoga stresa, lipidne peroksidacije i oštećenja DNK-a (19, 20). Među citosolnim klasama, GSTM1 (mu), GSTT1 (theta) i GSTP1 (pi) privukli su veliku pozornost u stomatološkim istraživanjima zbog izražene polimorfne varijabilnosti i implikacije u patofiziologiji različitih upalnih, neoplastičnih i degenerativnih bolesti (19, 20). Pretpostavlja se da poremećaji u funkciji GST-a, zbog genetskih delecija (npr. kod nositelja s *GSTM1* ili *GSTT1* deletiranim genotipovima), mogu ugroziti njihov zaštitni kapacitet čime pridonose pojačanom uništavanju tkiva i progresiji bolesti (19, 20).

U nekoliko studija autori su najprije istraživali povezanost između različitih GST polimorfizama i prisutnosti marginalnog parodontitisa (21 – 26). Njihovi nalazi istaknuli su važnost genetskih varijacija u GST enzimima, posebno *GSTM1, GSTT1* i *GSTP1*, u utjecaju na individualnu osjetljivost kad je riječ o kroničnom parodontitisu. Istraživanja su pokazala da ljudi koji imaju deletirane genotipove *GSTM1* i *GSTT1,* ili oni koji nose varijantni alel (G) na lokusu rs1695 *GSTP 1*, pokazuju kompromitiranu antioksidacijsku obranu, što ih čini osjetljivijima na oksidacijski stres i upalna oštećenja u parodontnim tkivima (21 – 26).

Iako marginalni i apikalni parodontitis imaju slične patološke značajke, nedostaju dokazi o utjecaju GST polimorfizama na razvoj AP-a. Zato je primarni cilj ove studije bio ispitati potencijalnu povezanost između *GSTT/GSTM* delecijskih polimorfizama i pojave apikalnog parodontitisa u populaciji pacijenata u dvama sveučilišnim centrima: Medicinskom fakultetu Sveučilišta u Banjoj Luci u Bosni i Hercegovini i Stomatološkom fakultetu Sveučilišta u Beogradu u Srbiji.

Materijali i metode

Ova studija je kliničko-laboratorijsko i opservacijsko istraživanje genetske asocijacije. Istraživanje je provedeno i prikazano u skladu s kontrolnim popisom za jačanje izvještavanja genetskih studija asocijacije (STREGA) (27) (dodatna tablica 1.) te prema smjernicama za izvještavanje laboratorijskih i opservacijskih studija u endodonciji (PRILE, PROBE) (28, 29).

Etička razmatranja

Protokol koji je primijenjen u ovom istraživanju u dvama centrima pregledala su i odobrila oba institucionalna etička povjerenstva za istraživanja (brojevi protokola: 36/12-2013 i 02-3-4-19-1-6/2022). Svi sudionici studije, odnosno njihovi roditelji ili zakonski skrbnici, potpisali su informirani pristanak, odnosno suglasnost. Nadalje, svi postupci provedeni su u skladu s etičkim standardima obaju odgovornih povjerenstava za ispitivanja na ljudima (Sveučilište u Banjoj Luci, BiH i Sveučilište u Beogradu, Srbija) te u skladu s Helsinškom deklaracijom iz 1975. godine, revidiranom 2002.

Table 1 Genotype frequencies of *GSTM* and *GSTT* deletion polymorphisms in patients with apical periodontitis and controls
Tablica 1. Učestalost genotipova delecijskih polimorfizama *GSTM1* i *GSTT1* kod bolesnika s apikalnim parodontitisom i u kontrolnoj skupini

Polymorphism • Polimorfizam	AP N = 200 (%)	Controls • kontrole N = 250 (%)	*P*-value • *P* vrijednost	OR (95% CI)
GSTM deletion • delecija				
GSTM +	69 (34,5)	154 (61,6)	ref	
GSTM -	131 (65,5)	96 (38,4)	<0,001	3,05 (2,07-4,9)
GSTT deletion • delecija				
GSTT +	99 (49,5)	212 (84,8)	ref	
GSTT -	101 (50,5)	38 (15,2)	<0,001	5,69 (3,66-8,86)
GSTM/GSTT deletion • delecija				
GSTM +/GSTT +	43 (21,5)	121 (48,4)	ref	
GSTM +/GSTT -	25 (12,5)	33 (13,2)	0,026	2,13 (1,14-3,98)
GSTM -/GSTT +	57 (28,5)	92 (36,8)	0,031	1,74 (1,08-2,82)
GSTM -/GSTT -	75 (37,5)	4 (1,6)	<0,001	52,76 (18,20-152,94)

AP – apical periodontitis • apikalni parodontitis; N – number of patients • broj pacijenata; OR – odds ratio • omjer mogućnosti; CI – confidence interval • interval pouzdanosti; GST – glutathione-S-transferase • glutation-S-transferaza

Sample description and collection of biological material

The present study involved 200 consecutive individuals exhibiting clinical, radiographic, and histopathological features of AP (30) who sought dental care at the University Dental Clinics in Banja Luka (50 patients) and Belgrade (150 patients) between January 2023 and January 2025. During routine clinical check-ups, information was gathered regarding the patients' general health and habits. The study only included the patients classified as ASA1 and/or 2 following the physical status classification proposed by the American Society of Anesthesiologists. Exclusion criteria were as follows: periodontally involved teeth with probing depths greater than 4 mm or vertical root fractures. In addition, individuals who were immunocompromised or had received antibiotics, antiviral medications, hormone therapy, or immunosuppressive treatments within three months prior to examination were not included in the study. The control group was comprised of 250 DNA samples taken from buccal swabs of healthy patients from the University Dental Clinics in Banja Luka (50 patients) and Belgrade (200 patients) with no history of AP.

As a source of genomic DNA, sterile buccal swabs were taken from each patient. All collected samples were refrigerated at 2–8°C overnight and stored at -70 °C before transport and DNA extraction.

DNA extraction, selection of genetic polymorphisms and genotyping

GSTM1 and *GSST1* genes were chosen as the vital components of the cellular antioxidant defense system, and the deletion polymorphisms were included to evaluate their potential role in susceptibility to AP. These variants were chosen based on earlier findings that were able to associate them with the presence of marginal periodontitis (21-26). Homozygous deletions of these genes result in complete loss of enzyme activity, thus leading to impaired detoxification of reactive oxygen species.

All laboratory procedures were performed in scientific laboratories at the Implant-Research Center, School of Den-

Opis uzorka i prikupljanje biološkog materijala

U ovoj studiji sudjelovalo je 200 pojedinaca s kliničkim, radiografskim i histopatološkim znakovima apikalnog parodontitisa (AP) (30) koji su zatražili stomatološku skrb u sveučilišnim stomatološkim klinikama u Banjoj Luci (50 pacijenata) i Beogradu (150 pacijenata) između siječnja 2023. i siječnja 2025. Tijekom rutinskih kliničkih pregleda prikupljene su informacije o općem zdravstvenom stanju i navikama pacijenata. Studija je obuhvaćala samo pacijente klasificirane kao ASA 1 i/ili 2 prema klasifikaciji fizičkoga statusa koju je predložilo Američko društvo anesteziologa.

Kriteriji za isključivanje bili su sljedeći: parodontalno zahvaćeni zubi s dubinom sondiranja većom od 4 mm ili vertikalne frakture korijena. U studiju također nisu bile uključene imunokompromitirane osobe te one koje su primale antibiotike, antivirusne lijekove, hormonsku terapiju ili imunosupresivne tretmane unutar tri mjeseca prije pregleda.

Kontrolnu skupinu činilo je 250 uzoraka DNK uzetoga bukalnim brisovima od zdravih ispitanika bez nalaza apikalnog parodontitisa sa sveučilišnih stomatoloških klinika u Banjoj Luci (50 pacijenata) i Beogradu (200 pacijenata).

Kao izvor genomskoga DNK korišteni su sterilni bukalni brisovi uzeti od svakog ispitanika. Svi prikupljeni uzorci preko noći su čuvani u hladnjaku na temperaturi između 2 i 8 °C, a zatim pohranjeni na -70 °C prije transporta i ekstrakcije DNK.

Ekstrakcija DNK, odabir genetskih polimorfizama i genotipizacija

Geni *GSTM1* i *GSST1* odabrani su kao vitalne komponente staničnoga antioksidacijskoga obrambenog sustava, a delecijski polimorfizmi uključeni su da bi se procijenila njihova potencijalna uloga u nastanku AP-a. Te varijante odabrane su na temelju ranijih otkrića koja su ih mogla povezati s marginalnim parodontitisom (21 – 26). Homozigotne delecije tih gena rezultiraju potpunim gubitkom enzimske aktivnosti, što završava oslabljenom detoksikacijom reaktivnih kisikovih vrsta.

Svi laboratorijski postupci provedeni su u znanstvenim laboratorijima u Implantološko-istraživačkom centru Stoma-

tal Medicine, and University of Belgrade, Serbia. The samples collected at the other center were appropriately stored and transported in a liquid nitrogen canister to the laboratory facilities. Genomic DNA that was used for the genotyping analysis was isolated from buccal cells collected using cotton swabs, as adopted in previous evaluations (31). The amount and purity of the DNA was identified by a BioSpec-nano UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan).

Genotyping of GSTM1 and GSTT1 deletion polymorphisms was conducted using multiplex PCR (32). Multiplex PCR mix (total volume 25 µL) was prepared using PCR Master Mix (2X) (Thermo Fisher Scientific) and contained 200 nmol /L of each primer, namely: for GSTT1 5'-TTCCTTACTG-GTCCTCACATCTC-3' and 5'-TCACCGGATCATGGC-CAGCA -3'; for GSTM1 5'-GAACTCCCTGAAAAGCTA-AAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3' (32) (Microsynth AG, Balgach, Switzerland) and 0.2 µg of genomic DNA. The amplification products were separated on 8% polyacrylamide (PAA) gels, then stained using ethidium bromide and exposed to UV light for visualization. Amplification of a segment of b-globin gene (110 bp) using primers 5'-ACACAACTGTGTTCAACTAGC-3' and 5'-CAACTTCATCCACGTTCACC-3' (33) served as an internal amplification control (IAC) in order to avoid false-negative results. Finally, GSTM1 null genotype (M1–/–) did not exhibit a 215 bp amplicon, while GSTT1 null genotype revealed no 480 bp amplification product.

Statistical analysis

SPSS Statistics for Windows Software, version 22.0 (IBM Corp, Armonk, NY, USA) and R statistical software (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) were used to carry out all calculations. In order to assess potential differences in categorical variables, the Pearson chi-square test and Fisher's exact test were performed. The Student's t-test or Mann–Whitney U-test were used to determine the statistical significance between numerical data. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) the values of which were then considered in order to estimate the risk of AP development with regard to the genotypes. The statistical analyses were two-sided, and p-values of 0.05 were perceived as statistically significant.

Results

Due to the nature of the genotyping method, it was not possible to determine the allelic distribution for the GSTM1 and GSTT1 deletion polymorphisms.

Among AP patients, 96 were male and 104 female, while the control group consisted of 110 male and 140 female participants. There was no statistical difference in gender distribution (Pearson's chi-square test, p = 0.446). The mean age of the AP group was 37.72 ± 12.89 years, and the control group was 35.60 ± 12.01 years. No significant difference in age distribution was observed (Student's t-test, p = 0.154).

tološkog fakulteta Sveučilišta u Beogradu, Srbija. Uzorci prikupljeni u drugom centru bili su odgovarajuće pohranjeni i transportirani u spremniku s tekućim dušikom u laboratorijske prostorije. Genomski DNK koji je korištena za genotipizaciju izoliran je iz bukalnih stanica prikupljenih pamučnim štapićima, prema protokolu korištenom u prijašnjim istraživanjima (31). Količina i čistoća DNK identificirane su BioSpec-nano UV-Vis spektrofotometrom (Shimadzu, Kyoto, Japan).

Genotipizacija delecijskih polimorfizama *GSTM1* i *GSTT1* provedena je korištenjem multipleks PCR-a (32). Multipleks PCR smjesa (ukupni volumen 25 µL) pripremljena je korištenjem PCR Master Mixa (2X) (Thermo Fisher Sci ntific) i sadržavala je 200 nmol/L svakog sastojka, i to za GSTT1 5'-TTCCTTACTGGTCCTCA-CATCTC-3' i 5'-TCACCGGATCATGGCCAGCA-3'; za GSTM1 5'-GAACTCCCTGAAAAGCTAAAGC-3' i 5'-GTTGGGCTCAAATATACGGTGG-3' (32) (Microsynth AG, Balgach, Švicarska) i 0,2 µg genomskoga DNK. Produkti amplifikacije odvojeni su na 8-postotnim poliakrilamidnim (PAA) gelovima, zatim obojeni etidijevim bromidom i vizualizirani pod UV-svjetlom. Amplifikacija segmenta β-globin gena (110 bp) korištenjem sastojaka 5'-ACACAACTGTGTTCAACTAGC-3' i 5'-CAACTTCATCCACGTTCACC-3' (33), poslužila je kao unutarnja kontrola amplifikacije (IAC) kako bi se izbjegli lažno negativni rezultati. Konačno, nulti genotip *GSTM 1* (M 1–/–) nije pokazao amplikon od 215 bp, a nulti genotip *GSTT 1* nije pokazao produkt amplifikacije od 480 bp.

Statistička analiza

Za sve izračune korišteni su SPSS Statistics for Windows, verzija 22.0 (IBM Corp, Armonk, NY, SAD) te R statistički softver (R Core Team, 2018. *R: Jezik i okruženje za statističko računanje*. R Foundation for Statistical Computing, Beč, Austrija). Da bi se procijenile potencijalne razlike u kategoričkim varijablama, primijenjeni su Pearsonov hi-kvadrat test i Fisherov egzaktni test. Za usporedbu numeričkih podataka korišten je Studentov t-test ili Mann-Whitneyjev U-test, ovisno o raspodjeli podataka. Bezuvjetna logistička regresija korištena je za izračun omjera mogućnosti (OR) i 95-postotnoga intervala pouzdanosti (CI) čije su vrijednosti zatim uzete u obzir da bi se procijenio rizik od pojave AP-a u odnosu na genotipove. Statističke analize bile su dvostrane, a p-vrijednosti manje od 0,05 smatrane su statistički značajnima.

Rezultati

Zbog prirode metode genotipizacije nije bilo moguće odrediti alelnu raspodjelu za delecijske polimorfizme *GSTM1* i *GSTT1*.

Među pacijentima s AP-om bilo je 96 muškaraca i 104 žene, a kontrolna skupina sastojala se od 110 muškaraca i 140 žena. Nije bilo statistički značajne razlike u spolnoj raspodjeli (Pearsonov hi-kvadrat test, p = 0,446). Prosječna dob AP skupine bila je 37,72 ± 12,89 godina, a kontrolne 35,60 ± 12,01 godina. Nije ustanovljena značajna razlika u dobnoj raspodjeli (Studentov t-test, p = 0,154).

The presence of *GSTM1* deletion was detected in 131 AP patients (65.5%) and 96 controls (38.4%). Furthermore, this genotype was associated with a 3.05-fold risk increase for developing AP (OR = 3.05, 95% CI = 2.07–4.49, p < 0.001; Table 1).

GSTT1 deletion was observed in 101 AP patients (50.5%) and 38 controls (13%), leading to a more prominent increase in the risk for the development of AP (OR = 5.69, 95% CI = 3.66–8.86, p < 0.001; Table 1).

In the combined analysis of both polymorphisms, the deletion of *GSTT1* in the presence of *GSTM1* affected the risk for AP development more notably (OR = 2.13, 95% CI = 1.14–3.98, p = 0.026) than vice versa (OR = 1.74, 95% CI = 1.08–2.82, p = 0.031; Table 1). Strikingly, 75 out of 200 patients with periapical lesions (37.5%) and 4 out of 250 controls (3.3%) exhibited the so-called double null genotype, i.e., deletions of both *GSTM1* and *GSTT1*. The co-occurrence of both deletions posed a significantly greater risk for AP development (OR = 52.76, 95% CI = 18.20–152.94, P < 0.001; Table 1).

Discussion

Recent scientific evidence clearly shows that AP should be considered as a multifactorial disease, highlighting the complexity of its underlying causes (1, 12, 13). While the polymicrobial infection of the pulp is undoubtedly the primary factor in its development, various environmental and predisposing factors also can significantly contribute to AP development (1, 12, 13). Exploring this issue, we aimed to assess the frequency of *GSTT* and *GSTM* deletion polymorphisms in patients with AP and to compare them with healthy individuals without a history of AP. It has been revealed that carriers of null *GSTT*, null *GSTM*, and double null *GSTT/GSTM* genotypes are significantly more susceptible to developing AP. Given the critical role of GST in maintaining normal tissue homeostasis (19, 20), these results strongly suggest that antioxidant defense is usually compromised in individuals inheriting these specific genotypes. Consequently, they are more vulnerable to oxidative stress and inflammatory damage affecting their periapical tissues.

The obtained findings are in alignment with previously reported results in the field of Periodontology (21-26). Namely, there have been a few studies that focused on investigating the role of different *GST* polymorphisms and susceptibility to the development of multiple forms of marginal periodontitis (21-26). Kim et al investigated the prevalence of the polymorphisms of genes Cytochrome *P450 (CYP) 1A1*, *CYP2E1*, and *GSTM1* that have a role in metabolic activation and detoxification of tobacco-derived substances in 115 individuals with periodontitis and 126 controls (22). The authors revealed that individuals with the *GSTM* allele were at a significant risk of developing periodontitis (OR = 2.1, 95% CI = 1.3–3.6) (22). Concolino et al (23) analyzed the association between *GSTT* and *GSTM* deletion polymorphisms and chronic and aggressive periodontitis cases (75 individuals) in a population of Caucasians. They reported there was a significant link between the *GSTM*-null genotype and both

Prisutnost delecije *GSTM1* otkrivena je kod 131 pacijenta s AP-om (65,5 %) i 96 u kontrolnoj skupini (38,4 %). Nadalje, taj je genotip bio povezan s 3,05 puta većim rizikom za pojavu AP-a (OR = 3,05, 95 % CI = 2,07 – 4,49, p < 0,001; tablica 1.).

Delecija *GSTT1* uočena je kod 101 pacijenta s AP-om (50,5 %) i 38 ispitanika iz kontrolne skupine (13 %), što je dovelo do izraženijeg povećanja rizika za nastanak AP-a (OR = 5,69, 95 % CI = 3,66 – 8,86, p < 0,001; tablica 1.).

U kombiniranoj analizi obaju polimorfizama, delecija *GSTT1* u prisutnosti *GSTM1* značajnije je utjecala na rizik od pojave AP-a (OR = 2,13; 95 % CI = 1,14 – 3,98; p = 0,026) nego obrnuta kombinacija (OR = 1,74; 95 % CI = 1,08 – 2,82; p = 0,031; tablica 1.).

Zanimljivo je da je 75 od 200 pacijenata s periapikalnim lezijama (37,5 %) i 4 od 250 ispitanika iz kontrolne skupine (3,3 %) pokazalo takozvani dvostruki nulti genotip, tj. istodobnu deleciju i *GSTM1* i *GSTT1*. Ta je kombinacija znatno rizičnija za pojavu AP-a (OR = 52,76; 95 % CI = 18,20 – 152,94; p < 0,001; tablica 1.).

Rasprava

Nedavni znanstveni dokazi jasno pokazuju da AP treba smatrati multifaktorijalnom bolešću, ističući složenost njegovih temeljnih uzroka (1, 12, 13). Iako je polimikrobna infekcija pulpe nedvojbeno primarni čimbenik u njegovu nastanku, različiti okolišni i predisponirajući čimbenici također mogu značajno pridonijeti pojavi AP-a (1, 12, 13). Istražujući to pitanje, cilj nam je bio procijeniti učestalost delecijskih polimorfizama *GSTT* i *GSTM* kod bolesnika s AP-om i usporediti ih sa zdravim osobama bez AP-a. Otkriveno je da su nositelji deletiranih genotipova za *GSTT*, *GSTM* i dvostruko deletiranih *GSTT/GSTM* znatno podložniji pojavi AP-a. S obzirom na ključnu ulogu GST-a u održavanju normalne homeostaze tkiva (19, 20), ti rezultati snažno upućuju na to da je antioksidacijska obrana obično ugrožena kod osoba koje nasljeđuju te specifične genotipove. Posljedično, osjetljiviji su na oksidacijski stres i upalna oštećenja koja utječu na njihova periapikalna tkiva.

Dobiveni nalazi u skladu su s već objavljenim rezultatima u području parodontologije (21 – 26). Naime, provedeno je nekoliko studija čiji su se autori usredotočili na istraživanje uloge različitih GST polimorfizama i sklonost prema pojavi višestrukih oblika marginalnog parodontitisa (21 – 26). Kim i suradnici istraživali su prevalenciju polimorfizama gena citokroma *P450 (CYP) 1A1*, *CYP2E1* i *GSTM1* koji imaju ulogu u metaboličkoj aktivaciji i detoksikaciji tvari izvedenih iz duhana kod 115 osoba s parodontitisom i 126 iz kontrolne skupine (22). Otkrili su da su osobe s alelom *GSTM* imale značajan rizik od pojave parodontitisa (OR = 2,1, 95 % CI = 1,3 – 3,6) (22). Concolino i suradnici (23) analizirali su povezanost između delecijskih polimorfizama za *GSTT* i *GSTM* te slučajeve kroničnog i agresivnog parodontitisa (75 pojedinaca) u populaciji bijelaca. Izvijestili su da postoji značajna veza između deletiranoga *GSTM* genotipa i kroničnih i agresivnih oblika parodontitisa. Uz to, oblici klasificirani kao agresivni korelirali su s dvostrukom deletiranom kom-

chronic and aggressive forms of periodontitis. In addition, the forms classified as aggressive correlated with the double null combination of *GSTM* and *GSTT* genes (23). Similarly, Arshad et al (24) found that the presence of both *GSTT* and the mutant allele (G) at rs1695 in *GSTP* significantly correlated with chronic periodontitis in a population of Pakistanis. As opposed to these data, a study conducted in a population of Czech Caucasians was not able to establish a strong link between *GST* gene variants and an increased risk of developing periodontitis (28). Although the current findings are promising, further research is required in order to confirm the impact of *GST* polymorphism on the development of various types of periodontal diseases.

Despite the growing number of genetic association studies in Endodontology (34), these specific polymorphisms have not been thoroughly examined. In contrast, recent research has focused on the role of other genes related to oxidative stress imbalance (35-37). By employing logistic regression analysis, Meyfarth et al (35) found that SNPs in nitric oxide synthase (rs2297518 and rs2779249) were not associated with persistent AP and SNP-SNP interactions in a Brazilian population. Da Silva Guimarães et al (36) reported that superoxide dismutase (SOD) gene polymorphisms [*SOD2* (rs5746136, rs4880, and rs10370) and *SOD3* (rs2855262 and rs13306703)] can influence oral health-related quality of life response in individuals with asymptomatic periapical periodontitis. Finally, Karataş et al (37) evaluated the correlation between polymorphisms in catechol-O-methyl transferase (*COMT*), opioid receptor Mu 1(*OPRM1*), and serotonin receptor genes [5-Hydroxytryptamine Receptor 1A (*5HT1A*), (*5HT2A*), and (*5HTR3B*)] and recorded postoperative pain intensity levels after undergoing root canal treatment. It was concluded that the SNPs assessed in relation to *COMT, OPRM1, 5HT1A, 5HT2A,* and *5HTR3B* genes could not be linked to the intensity of postoperative pain. However, additional investigations in this field are essential to establish a definitive conclusion regarding a potential association.

The most prominent limitation of the current study is the restricted number of SNPs that were investigated. Recently, Petty et al (38) were the first to perform a genome-wide association study (GWAS) of apical periodontitis in a population that was large and well-characterized. Namely, the authors provided ample evidence pointing to host-mediated genetic effects on susceptibility to developing apical periodontitis. It has been stated that genome-wide analyses have identified a top-associated variant situated near the RAP1 GTPase-activating protein (RAP1GAP), which functions as an inhibitor of Ras-related protein 1 (RAP1) (38). It is crucial in both physiological and inflammatory pathways, including macrophage-mediated phagocytosis, chemokine-driven cell adhesion, and leukocyte trafficking, as well as the homing of lymphocytes and dendritic cells. Moreover, it also facilitates cellular attachment to extracellular matrix components such as fibronectin, fibrinogen, collagen, and laminin (39). Furthermore, the authors stated that the associations identified by GWAS are not to be explicitly linked to disease causation. Instead, follow-up studies focusing on functional implications should be prioritized in future research (39).

binacijom gena *GSTM* i *GSTT* (23). Slično tomu, Arshad i suradnici (24) otkrili su da prisutnost i *GSTT-a* i mutantnoga alela (G) na rs1695 u *GSTP-u* značajno korelira s kroničnim parodontitisom u populaciji Pakistanaca. Suprotno tim podatcima, u studiji provedenoj na češkoj populaciji bijelaca nije se uspjela ustanoviti snažna veza između varijanti gena *GST* i povećanog rizika od pojave parodontitisa (28). Iako trenutačni nalazi obećavaju, potrebna su daljnja istraživanja kako bi se potvrdio utjecaj polimorfizma GST na pojavu različitih vrsta parodontalnih bolesti.

Unatoč sve većem broju studija o genetskoj asocijaciji u endodontologiji (34), ti specifični polimorfizmi nisu temeljito ispitani. Suprotno tomu, autori nedavnih istraživanja usredotočili su se na ulogu drugih gena povezanih s neravnotežom oksidacijskoga stresa (35 – 37). Korištenjem logističke regresijske analize, Meyfarth i suradnici (35) otkrili su da polimorfizmi na razini jednog nukleotida (SNP) u sintazi dušikova oksida (rs2297518 i rs2779249) nisu povezani s perzistentnim AP i SNP-SNP interakcijama u brazilskoj populaciji. Da Silva Guimarães i suradnici (36) izvijestili su da polimorfizmi gena superoksid-dismutaze [SOD] *(*SOD2* (rs5746136, rs4880 i rs10370) i *SOD3* (rs2855262 i rs13306703)] mogu utjecati na kvalitetu života povezanu s oralnim zdravljem kod osoba s asimptomatskim periapikalnim parodontitisom. Konačno, Karataş i suradnici (37) procijenili su korelaciju između polimorfizama u genima katehol-O-metil transferaze (*COMT*), opioidnog receptora Mu 1 (*OPRM1*) i serotoninskih receptora [5-hidroksitriptaminski receptor 1A *(5HT1A), (5HT2A)* i *(5HTR3B)*] te zabilježili razinu intenziteta postoperativne boli poslije liječenja korijenskog kanala. Zaključeno je da SNP-ovi u genima za *COMT, OPRM1, 5HT1A, 5HT2A* i *5HTR3B* ne mogu biti povezani s intenzitetom postoperativne boli. No dodatna istraživanja u ovom području prijeko su potrebna za donošenje konačnog zaključka o potencijalnoj povezanosti. Najistaknutije ograničenje ove studije jest ograničen broj istraživanih SNP-ova. Nedavno su Petty i suradnici (38) prvi proveli studiju asocijacije na razini cijelog genoma (GWAS) apikalnog parodontitisa u populaciji koja je bila velika i dobro karakterizirana. Naime, autori su pružili obilje dokaza koji upućuju na genetske učinke posredovane domaćinom na sklonost prema pojavi apikalnog parodontitisa. Navedeno je da su analize na razini cijeloga genoma identificirale varijantu povezanu s proteinom koji aktivira RAP1 GTP-ase (RAP1GAP), a funkcionira kao inhibitor proteina 1 povezanog s Ras-om (RAP1) (38). Navedena molekula ključna je i u fiziološkim i u upalnim putovima, uključujući fagocitozu posredovanu makrofagima, staničnu adheziju potaknutu kemokinima i promet leukocita, te usmjeravanje limfocita i dendritičnih stanica. Štoviše, također olakšava stanično vezanje za komponente izvanstanične matrice kao što su fibronektin, fibrinogen, kolagen i laminin (39). Nadalje, autori su naveli da se povezanosti identificirane GWAS-om ne smiju eksplicitno povezivati s uzročnošću bolesti. Umjesto toga, u budućim istraživanjima treba dati prioritet studijama praćenja usmjerenima na funkcionalne implikacije (39).

Također postoji nekoliko važnih prednosti ove studije. Naime, provedena je i prijavljena u skladu sa smjernicama

There are also several important strengths of this study. This study was performed and reported according to STREGA guidelines for genetic association studies (27). Moreover, the authors followed all recommendations for primary clinical investigations proposed in the latest umbrella review related to this topic (14). In addition, this is the first two-center study that merged the investigated populations from two university centers (200 individuals with AP and 250 healthy controls, in total). As a result, the primary benefits of this study included enhancing statistical power and increasing the generalizability of the observed results.

Insights into individual genetic variations hold promise for advancing precision-based dental care, thus allowing for more individualized prevention and treatment approaches. Detection of gene polymorphisms linked to exaggerated inflammatory responses may help clinicians estimate a patient's risk of developing or progressing oral diseases. Such genetic insights could also support the customization of therapeutic strategies, thus ensuring better clinical efficacy and a reduced likelihood of disease relapse. Considering the vital role of GSTs in redox balance and tissue health, it is imperative that individuals with compromised antioxidant defenses, particularly those at a heightened risk of cancer, undergo targeted screening for AP. This necessity underscores a significant area for future translational research and preventive strategies.

Conclusion

The carriers of null *GSTT*, null *GSTM*, and double null *GSTT/GSTM* genotypes are more susceptible to the development of disease in the investigated two-center population. Further genetic association studies involving a broader range of relevant gene polymorphisms, their functional implications, and larger population samples are required to validate the significance of the polymorphisms examined in this study.

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

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STREGA-e za studije genetske asocijacije (27). Štoviše, autori su slijedili sve preporuke za primarna klinička istraživanja predložene u najnovijem krovnom pregledu vezanom uz tu temu (14). Osim toga, ovo je prva studija u dvama centrima koja je spojila ispitivane populacije iz dvaju sveučilišnih središta (ukupno 200 osoba s AP-om i 250 zdravih osoba u kontroli). Kao rezultat toga, primarne koristi od ove studije uključivale su povećanje statističke snage i povećanje generalizacije promatranih rezultata.

Uvidi u individualne genetske varijacije obećavaju napredak u preciznoj stomatološkoj skrbi, omogućujući individualizaciju pristupa prevenciji i liječenju. Detekcija genskih polimorfizama povezanih s pretjeranim upalnim odgovorima može pomoći kliničarima u procjeni pacijentova rizika od pojave ili napredovanja oralnih bolesti. Takvi genetski uvidi također bi mogli poduprijeti prilagodbu terapijskih strategija i osigurati bolju kliničku učinkovitost i smanjenu vjerojatnost recidiva bolesti. S obzirom na vitalnu ulogu GST-a u redoks ravnoteži i zdravlju tkiva, nužno je da se osobe s kompromitiranom antioksidacijskom obranom, posebno one s povećanim rizikom od raka, podvrgnu ciljanom probiru na AP. Ta nužnost ističe važno područje za buduća translacijska istraživanja i preventivne strategije.

Zaključak

Nositelji deletiranih genotipova za *GSTT*, *GSTM* i dvostruko deletiranih *GSTT/GSTM* podložniji su pojavi bolesti u ispitivanoj populaciji iz dvaju centara. Potrebna su daljnja istraživanja genetske asocijacije koja uključuju širi raspon relevantnih genskih polimorfizama, njihove funkcionalne implikacije i više dodatnih populacija kako bi se potvrdila značajnost polimorfizama ispitivanih u ovoj studiji.

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Sažetak

Svrha rada: Primarni cilj ove studije bio je ispitati potencijalnu povezanost između delecijskih polimorfizama za glutation S-transferazu (*GSTT1/GSTM1*) i pojave apikalnog parodontitisa (AP) u populaciji pacijenata u dvama sveučilišnim centrima: Medicinskom fakultetu Sveučilišta u Banjoj Luci u Bosni i Hercegovini i Stomatološkom fakultetu Sveučilišta u Beogradu u Srbiji. **Materijali i metode:** U studiji je sudjelovalo 200 pacijenata s AP-om u eksperimentalnoj skupini i 250 zdravih osoba bez AP-a u kontrolnoj skupini. Kao izvor genomskoga DNK, od svakog pacijenta uzeti su sterilni brisovi bukalne sluznice. Genotipizacija delecijskih polimorfizama *GSTM1* i *GSTT1* provedena je multipleksnom lančanom reakcijom polimeraze (PCR). Rizik od nastanka AP-a, s obzirom na genotipove, procijenjen je na temelju omjera mogućnosti (OR) i 95-postotnog intervala pouzdanosti (CI) koji su izračunati bezuvjetnom logističkom regresijom. **Rezultati:** Postojale su značajne razlike u demografskim karakteristikama između ispitivanih skupina (p = 0,446, p = 0,154). Deletirane verzije *GSTM 1* i *GSTT 1* bile su povezane s 3,05 puta većim, odnosno 5,69 puta većim rizikom za pojavu AP-a (OR = 3,05, 95 % CI = 2,07 – 4,49, OR = 5,69, 95 % CI = 3,66 – 8,86, p < 0,001, p < 0,001). Istodobna pojava obiju deletiranih verzija bila je značajno veći rizik za nastanak AP-a (OR = 52,76, 95 % CI = 18,20 – 152,94, P < 0,001). **Zaključci:** Nositelji deletiranih genotipova za *GSTT* i *GSTM* te dvostruko deletiranih za *GSTT/GSTM* podložniji su pojavi AP-a u populacijama ispitivanima u dvama centrima.

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