ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta stomatol Croat. 2022;56(4):387-394.
DOI: 10.15644/asc56/4/5

ORIGINAL SCIENTIFIC PAPER
IZVORNI ZNANSTVENI RAD

Nikolija Savčić[1], Damir Henjaš[1], Marija Jezdić[2], Ana Đinić Krasavčević[2], Iva Milinković[2]

Porphyromonas Gingivalis in Different Peri-Implant Conditions: a Pilot Cross - Sectional Study

Porphyromonas gingivalis *kod različitih periimplantatnih stanja: presječno pilot-istraživanje*

[1] Student, School of Dental Medicine, University of Belgrade, Belgrade, Serbia
Student Stomatološkog fakulteta Sveučilišta u Beogradu, Srbija
[2] Department of Periodontology and Oral medicine, School of Dental Medicine, University of Belgrade, Belgrade, Serbia
Zavod za parodontologiju i oralnu medicinu Stomatološkog fakulteta Sveučilišta u Beogradu, Srbija

Abstract

Objective: To assess the potential correlation between clinical peri-implant parameters and the presence of *Porphyromonas gingivalis* in different peri-implant conditions. **Material and methods:** The study included 30 patients from the Department of Periodontology and Oral Medicine divided into three equal groups, defined according to the following diagnoses: peri-implantitis (PI), peri-implant mucositis (PM), and peri-implant health (HI). Clinical parameters such as peri-implant probing depth (PPD), bleeding on probing (BOP), suppuration on probing (SUP), and plaque index (PI) were recorded. The samples of peri-implant crevicular fluid were collected and relative levels of *Porphyromonas gingivalis* were analyzed using *Real-Time PCR (Reverse transcriptase – real-time polymerase chain reaction)*. **Results:** The mean patient age was 51.33 ± 12.61 years. The mean value for *Porphyromonas ginigvalis* relative level was higher in the PI group (14.80 ±31.51) compared to PM and HI group (0.48 ± 1.34 and 0.06 ± 0.12, respectively). This parameter significantly differed between PI and HI (p=0.012), as well as PI and PM (p=0.049). The mean whole mouth probing pocket depth (PPD) in the PI group (4.02mm ± 0.77mm) was also significantly greater compared to the other two groups (3.39mm ± 0.52mm in PM and 1.97mm ± 0.54mm in the HI group. The SUP in the PI (mean value of 18%) group was more frequent than in PM (mean value of 14%) while the SUP was not detected in the HI group. The correlation between clinical parameters and relative levels of *Porphyromonas gingivalis* was not significant. **Conclusions:** The findings of this research indicate higher relative levels of *Porphyromonas gingivalis* in peri-implant lesions, especially in peri-implantitis.

Received: July 20, 2022
Accepted: October 14, 2022

Address for correspondence
Ana Đinić Krasavčević
University of Belgrade
School of Dental Medicine
Department of Periodontology and Oral Medicine
Dr Subotića 4, 11 000 Belgrade, Serbia
ana.djinic@gmail.com

MeSH Terms: Porphyromonas gingivalis; Peri-implantitis; Periodontal Diseases; Mucositis
Author Keywords: Peri-implant Mucositis; Peri-implant Health

Ana Đinić Krasavčević https://orcid.org/0000-0001-9439-0529
Marija Jezdić https://orcid.org/0000-0003-1379-6717
Iva Milinković https://orcid.org/0000-0001-5664-7848

Introduction

The outcomes of dental implant treatment are evaluated by means of survival and success rate. While survival rate means that dental implant is still present in the mouth, the success rate additionally assumes the absence of any type of biological and/or mechanical complications, alongside with accomplished function and esthetics (1). Although nowadays high implant survival and success rates could be achieved in even challenging clinical scenarios (2), long-term success rates of dental implant treatment could be compromised by the inflammatory processes in the surrounding peri-implant tissues. Inflammation around dental implants causes biological complications that manifest in the following two conditions: peri-implant mucositis and peri-implantitis (3, 4).

Peri-implant mucositis represents the reversible inflammation of peri-implant soft tissues (Figure 1), while the clinical signs of peri-implantitis are characterized by soft tis-

Uvod

Ishodi liječenja dentalnim implantatima procjenjuju se prema preživljenju i stopi uspješnosti. Dok stopa preživljenja znači da je dentalni implantat još uvijek u ustima, stopom uspješnosti smatra se nepostojanje bilo koje vrste bioloških i/ili mehaničkih komplikacija, uz ostvarenu funkciju i estetiku (1). Iako se danas mogu postići visoke stope preživljenja i uspješnosti implantata čak i u izazovnim kliničkim slučajevima (2), dugoročne stope uspješnosti liječenja dentalnim implantatima mogu biti ugrožene upalnim procesima u okolnim periimplantatnim tkivima. Upala oko dentalnih implantata uzrokuje biološke komplikacije koje se manifestiraju kao periimplantatni mukozitis i periimplantitis (3, 4).

Periimplantatni mukozitis reverzibilna je upala periimplantantnih mekih tkiva (slika 1.), a periimplantitis je upala mekih tkiva praćena progresivnim gubitkom marginalne kosti oko implantata (slika 2.) (5, 6). Periimplantantni muko-

sue inflammation, accompanied by progressive peri-implant marginal bone loss (Figure 2) (5, 6). Peri-implant mucositis is regarded as the predecessor of peri-implantitis, since, if not treated, it can easily progress into peri-implantitis.

Even though different potential inducers of inflammatory reaction in peri-implant compartment were described (7), the main etiological factor causing biological complications is the bacterial biofilm in the peri-implant area. Importantly, periodontal pathogens persisted for a long period in the oral cavity of edentulous subjects with a history of periodontitis, even in the absence of other hard subgingival surfaces in the mouth (8). Additionally, the fast microbial colonization of the implant surface after the installation has been demonstrated, showing a similar composition of bacterial species at implant and tooth sites three months later (9). It should also be mentioned that periodontitis and peri-implantitis exhibit some similarities concerning the etiology, pathogenesis, risk factors, diagnosis, and treatment (10). Nonetheless, there are differences, mostly in terms of disease progression and response to treatment. Peri-implantitis shows a non-linear and aggressive progression, developing faster than the periodontal disease (11).

As mentioned above, the main etiological factor in both peri-implant diseases is oral biofilm. Its formation starts around 30 minutes after the implant placement, and within two weeks the polymicrobial flora harboring healthy implants becomes similar to the flora surrounding natural teeth in the same mouth (12).

In peri-implantitis, the biofilm structure changes towards higher counts of more pathogenic microorganisms such as *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum,* and *Aggregatibacter actinomycetemcomitans* (13).

Among all these microorganisms, *Porphiromonas gingivalis* is the most common pathogen belonging to the red complex defined by Socransky (14). It is a Gram-negative, anaerobic, and asaccharolytic rod referred to be the keystone pathogen, which causes dysbiosis due to its ability to alter normal microbiota. This microorganism expresses virulence factors such as capsules, fimbriae, and proteases (gingipains). Capsule and fimbriae take place in facilitating colonization by adhering to periodontal epithelial cells, with the capsule helping co-aggregation with other pathogens and inducing anti-phagocytic activity. Gingipains are responsible for resistance to the host defense mechanisms (15, 16, 17, 18).

Even today, the pathogenesis of peri-implantitis is not completely understood, the bacterial peri-implant flora has not yet been fully studied, and the treatment guidelines have not been established. With regard to the currently available information in the literature, the objectives of this research were to evaluate the potential correlation between clinical peri-implant parameters and the presence of *Porphyromonas gingivalis*.

Material and methods

Patient population

This cross-sectional pilot study included patients recruited at the Department of Periodontology and Oral Medi-

zitis smatra se pretečom periimplantitisa, s obzirom na to da, ako se ne liječi, može lako prijeći u periimplantitis.

Iako su opisani različiti potencijalni induktori upalne reakcije oko implantata (7), glavni etiološki čimbenik koji izaziva biološke komplikacije jest bakterijski biofilm u periimplantantnom području. Važno je da su parodontni patogeni dugo postojali u usnoj šupljini bezubih subjekata s poviješću parodontitisa, čak i u nedostatku drugih tvrdih subgingivnih površina u ustima (8). Dodatno, dokazana je brza mikrobna kolonizacija površine implantata nakon ugradnje, pokazujući sličan sastav bakterijskih vrsta na mjestu implantata i zuba tri mjeseca poslije (9). Također treba spomenuti da parodontitis i periimplantitis pokazuju neke sličnosti kada je riječ o etiologiji, patogenezi, čimbenicima rizika, dijagnozi i liječenju (10). Unatoč tomu postoje razlike, uglavnom u vezi s napredovanjem bolesti i odgovorom na liječenje. Periimplantitis pokazuje nelinearnu i agresivnu progresiju i razvija se brže od parodontne bolesti (11).

Kao što je spomenuto, glavni etiološki čimbenik u objema periimplantatnim bolestima je oralni biofilm. Njegovo stvaranje počinje tridesetak minuta poslije ugradnje implantata, a unutar dva tjedna polimikrobna flora oko zdravih implantata postaje slična flori koja okružuje prirodne zube u istim ustima (12).

U slučaju periimplantitisa mijenja se struktura biofilma prema većem broju patogenih mikroorganizama kao što su *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum i Aggregatibacter actinomycetemcomitans* (13).

Među svim tim mikroorganizmima, *Porphiromonas gingivalis* najčešći je patogen koji pripada crvenome kompleksu definiranome prema Socranskyju (14). Radi se o gram-negativnom, anaerobnom štapiću koji se smatra glavnim patogenom zbog svojega svojstva da mijenja normalnu mikrobiotu, uzrokujući disbiozu. Taj mikroorganizam ima čimbenike virulencije kao što su kapsule, fimbrije i proteaze (gingipaini). Kapsula i fimbrije sudjeluju u olakšavanju kolonizacije prianjanjem na epitelne stanice parodonta, pri čemu kapsula pomaže koagregaciju s drugim patogenima i inducira antifagocitnu aktivnost. Gingipaini su odgovorni za otpornost na obrambene mehanizme domaćina (15, 16, 17, 18).

Patogeneza periimplantitisa ni danas nije u cijelosti razjašnjena, bakterijska periimplantatna flora još nije potpuno proučena, a smjernice liječenja nisu utvrđene. S obzirom na trenutačno dostupne informacije u literaturi, ciljevi ovog istraživanja bili su procijeniti potencijalnu korelaciju između kliničkih periimplantantnih parametara i prisutnosti bakterije *Porphyromonas gingivalis.*

Materijal i metode

Populacija pacijenata

Ovo presječno pilot-istraživanje obuhvaćalo je pacijente Zavoda za parodontologiju i oralnu medicinu Stomatološ-

cine, School of Dental Medicine, University of Belgrade. The present research was approved by the Ethics Committee of the School of Dental Medicine, prior to study initiation (no 36/15). The study was conducted in full accordance with the 2013 revision of the Helsinki Declaration of 1975.

The subjects included in the study were systemically healthy individuals at least 18 years of age, non-smokers or light smokers (up to 10 cigarettes per day). The patients who were enrolled earlier had received dental implants at the Department of Periodontology and Oral Medicine, after which they were coming for routine check-ups or spontaneous visits. All participants included had dental implants for at least one year in function. All types of titanium endosteal implants and prosthetic reconstructions have been included. Zirconium, pterygoid and zygomatic implants have not been considered. In case more than one implant was present, only the dental implant with the most severe clinical and radiological presentation was included.

The exclusion criteria were pregnancy or lactation, systematic diseases or drug therapy that affected the periodontium and bone metabolism, periodontal/peri-implant therapy in the last 6 months, local/ systematic usage of antimicrobial agents in the last 6 months, and usage of anti-plaque mouth wash in the last month.

Patients were divided into three groups according to the Classification of peri-implant conditions and diseases: peri-implantitis (PI), peri-implant mucositis (PM), and peri-implant health (HI).

The diagnosis of peri-implant health required: absence of clinical signs of inflammation; the absence of bleeding and/or suppuration on gentle probing; no increase in probing depth compared to previous examinations; the absence of bone loss beyond crestal bone level changes resulting from initial bone remodeling (19).

The diagnosis of peri-implant mucositis required: the presence of bleeding and/or suppuration on gentle probing with or without increased probing depth compared to previous examinations; the absence of continuing bone loss (observed on a radiograph): the absence of loss beyond crestal bone level changes resulting from initial bone remodeling (5) (Figure 1).

The diagnosis of peri-implantitis required: the presence of bleeding and/or suppuration on gentle probing; an increased probing depth compared to previous examinations; the presence of bone loss beyond crestal bone level changes resulting from initial bone remodeling.

In the absence of previous examination, the data diagnosis of peri-implantitis was based on: the presence of bleeding and/or suppuration on gentle probing; probing depths of ≥6 mm; bone levels ≥3 mm apical of the most coronal portion of the intraosseous part of the implant (6) (Figure 2).

Clinical parameters and microbiological sampling

During the same appointment, after the accurate diagnosis had been made, the following clinical parameters were recorded and analyzed: peri-implant probing depth (PPD), bleeding on probing (BOP), suppuration on probing (SUP), and plaque index (PI).

kog fakulteta Sveučilišta u Beogradu. Odobrilo ga je Etičko povjerenstvo Stomatološkog fakulteta prije početka istraživanja (br. 36/15). Istraživanje je provedeno u skladu s revizijom Helsinške deklaracije iz 1975.

Ispitanici uključeni u istraživanje bili su sistemski zdravi pojedinci u dobi od najmanje 18 godina, nepušači ili lagani pušači (do 10 cigareta na dan). Prije upisani pacijenti dobili su dentalne implantate u Zavodu za parodontologiju i oralnu medicinu, nakon čega su dolazili na redovite kontrole ili spontane posjete. Svi sudionici imali su dentalne implantate u funkciji najmanje godinu dana. Uključene su sve vrste titanijevih enosealnih implantata i protetičkih rekonstrukcija. Cirkonij-oksidni, pterigoidni i zigomatični implantati nisu uzeti u obzir. Ako je bilo ugrađeno više od jednoga implantata, uključen je samo onaj s najtežom kliničkom i radiološkom prezentacijom.

Kriteriji za isključivanje bili su trudnoća ili dojenje, sistemske bolesti ili terapija lijekovima koji su utjecali na parodont i metabolizam kostiju, parodontološka terapija u posljednjih 6 mjeseci, lokalna/sistemska primjena antimikrobnih sredstava u posljednjih 6 mjeseci i primjena antimikrobnih lijekova – ispiranje usta sredstvima protiv plaka u posljednjih mjesec dana.

Pacijenti su podijeljeni u tri skupine prema Klasifikaciji periimplantantnih stanja i bolesti: periimplantitis (PI), periimplantatni mukozitis (PM) i zdravo periimplantatno tkivo (HI).

Dijagnoza zdravoga periimplantatnog tkiva uključivala je odsutnost kliničkih znakova upale, odsutnost krvarenja i/ili gnojenja pri nježnom sondiranju, nije smjelo biti povećanja dubine sondiranja u usporedbi s prethodnim pregledima, nije smjelo biti gubitka koštane mase osim promjena razine krestalne kosti kao rezultat početnoga koštanog preoblikovanja (19).

Dijagnoza periimplantantnog mukozitisa zahtijevala je krvarenje i/ili gnojenje pri nježnom sondiranju s povećanom dubinom sondiranja ili bez njega u usporedbi s prethodnim pregledima, nije smjelo biti kontinuiranoga gubitka koštane mase (uočeno na rendgenskoj snimci), te gubitka kosti, osim razine krestalne kosti, koja je posljedica početnoga koštanog preoblikovanja (5) (slika 1.).

Dijagnoza periimplantitisa zahtijevala je krvarenje i/ili gnojenje pri nježnom sondiranju, povećanu dubinu sondiranja u usporedbi s prethodnim pregledima, gubitak koštane mase osim promjena razine krestalne kosti kao rezultat početnoga koštanoga preoblikovanja.

U nedostatku podataka o prethodnom pregledu dijagnoza periimplantitisa temeljila se na krvarenju i/ili gnojenju pri nježnom sondiranju, dubini sondiranja ≥ 6 mm; razini kosti ≥ 3 mm apikalno od najkoronalnijega dijela intraosealnoga dijela implantata (6) (slika 2.)

Klinički parametri i mikrobiološko uzimanje uzoraka

Tijekom istog pregleda, nakon što je postavljena dijagnoza, zabilježeni su i analizirani sljedeći klinički parametri: dubina sondiranja oko implantata (PPD), krvarenje pri sondiranju (BOP), gnojenje pri sondiranju (SUP) i indeks plaka (PI).

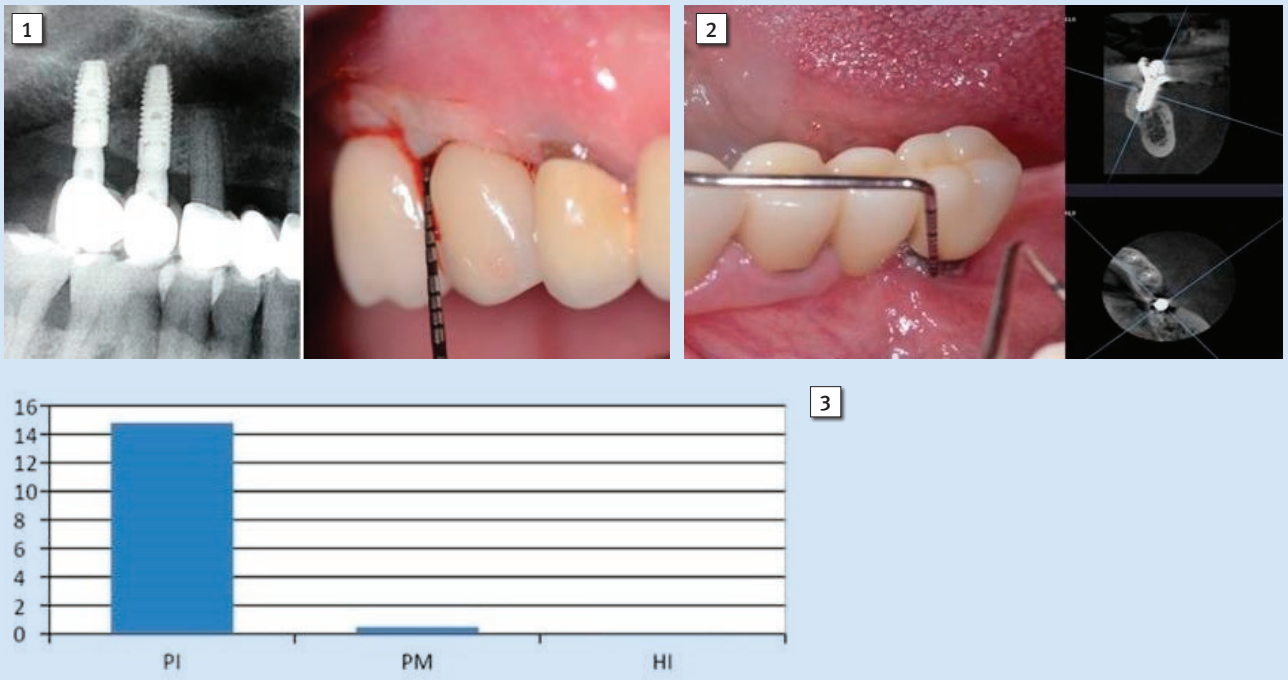

Figure 1 Clinical photography combined with x-ray represents inflammation of peri-implant soft tissue in peri-implant mucositis without bone loss.
Slika 1. Klinička fotografija u kombinaciji s rendgenskim snimkom predstavlja upalu periimplantnog mekog tkiva u periimplantnom mukozitisu bez gubitka koštane mase.
Figure 2 Clinical photography combined with CBCT slices represents a bone loss in peri-implantitis.
Slika 2. Klinička fotografija u kombinaciji s CBCT rezovima predstavlja gubitak kosti kod periimplantitisa.
Figure 3 Mean values for the relative level of *Porphyromonas gingivalis* in groups.
Slika 3. Srednje vrijednosti za relativnu razinu *Porphyromonas gingivalis* u skupinama.

Clinical parameters were measured at six sites per implant using a periodontal probe (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). These parameters were recorded in periodontal charts.

During the same visit, after the clinical examination, the selected implant site was isolated using cotton rolls in order to perform the microbiological sampling. Sampling was conducted by means of 3 paper points (size 30) that were inserted into the peri-implant sulcus/pocket until resistance was met, and left for a period of 30 seconds. The procedure was repeated to obtain a control sample. Paper points were stored inside sterile Eppendorf tubes that contained RNA stabilization solution (RNAlater Stabilization Solution, Thermo Fisher Scientific), then refrigerated overnight (2–8°C), after which the solution was removed and the samples were stored at –80°C. Further analyses were made in the Laboratory for Basic research, School of Dental Medicine, University of Belgrade, by means of Real-Time PCR (Reverse transcriptase – real-time polymerase chain reaction). The method utilized specific primers and implied multiplication of the bacterial genome. In separated tubes, the human genome was also multiplicated, as a control group. The results of this analysis were reported as relative levels of microorganisms- RQ value (relative quantity).

Statistical analysis

The statistical package (SPSS software package, version 22.0; SPSS Inc.) was used for all data analyses. Descriptive

Klinički parametri mjereni su na šest mjesta na implantatu s pomoću parodontološke sonde (meziobukalno, sredina bukalno, distobukalno, mezio-lingvalno, sredina lingvalno, distolingvalno). Ti su parametri zabilježeni u parodontološkim kartonima.

U istom posjetu, nakon kliničkoga pregleda, odabrano mjesto oko implantata izolirano je svitcima staničevine kako bi se obavilo mikrobiološko uzorkovanje. To je učinjeno s pomoću triju papirnatih štapića (veličine 30) koji su umetnuti u sulkus/džep oko implantata sve dok se nije naišlo na otpor i ostavljeni su 30 sekunda. Postupak je ponovljen da bi se dobio kontrolni uzorak. Papirnati štapići čuvani su u sterilnim Eppendorfovim epruvetama koje su sadržavale stabilizacijsku otopinu RNK (RNAlater Stabilization Solution, Thermo Fisher Scientific), zatim su ohlađene preko noći (2 – 8 °C), nakon čega je otopina uklonjena, a uzorci pohranjeni na -80 °C. Daljnje analize nastavljene su u Laboratoriju za temeljna istraživanja Stomatološkog fakulteta Sveučilišta u Beogradu, uz pomoć Real-Time PCR-a (Reverse transcriptase – real-time polymerase lančana reakcija). Metoda je obuhvaćala specifične početnice i podrazumijevala umnažanje bakterijskog genoma. U odvojenim epruvetama, kao kontrolna skupina, umnožen je i ljudski genom. Rezultati ove analize prikazani su kao relativne razine mikroorganizama – RQ vrijednost (relativna količina).

Statistička analiza

Za sve analize podataka korišten je statistički paket (programski paket SPSS, verzija 22.0; SPSS Inc.). Deskriptivna

statistics was presented as mean ± standard deviation, minimum and maximum. Comparisons between every two groups individually were performed by the Mann-Whitney test. The Spearman's rank correlation coefficient (ρ) was used to assess the relationship between clinical parameters and relative levels of *Porphiromonas gingivalis.* The significance was set at *p<0.05.*

Results

The present study included 30 patients with 30 inserted dental implants. Patient groups (PI, PM, HI) were balanced and each group consisted of 10 subjects. Subjects aged between 30 and 73 years, with mean patient age of 51.33 ± 12.61 years. The mean, standard deviation (SD), minimum (min), and maximum (max) values of clinical parameters for the assessed groups are presented in Table 1.

Mean PPD was the greatest in the PI group compared to the other two groups, as shown in Table 1. This parameter was significantly different among all three groups. The *p* values for examined clinical parameters among all groups are presented in Table 2; each group was compared with the other two groups.

The BOP and PI were the greatest in the PM group (Table 1). A significant difference in BOP was obtained between all groups, while in the PI parameter statistical significance was expressed between groups PI and HI and PM and HI (Table 2).

The SUP was more frequent in the PI group than in the PM group (Table 1), while it was not detected in the HI group. This parameter did not show a statistically signifi-

statistika prikazana je kao srednja vrijednost ± standardna devijacija, minimum i maksimum. Usporedbe između svake dvije skupine pojedinačno provedene su Mann-Whitneyjevim testom. Spearmanov koeficijent korelacije (ρ) korišten je za procjenu odnosa između kliničkih parametara i relativnih razina bakterije *Porphiromonas gingivalis*. Značajnost je postavljena na p < 0,05.

Rezultati

U ovo je istraživanje bilo uključeno 30 pacijenata s 30 ugrađenih dentalnih implantata. Skupine pacijenata (PI, PM, HI) bile su uravnotežene i svaka se sastojala od 10 ispitanika. Ispitanici su bili u dobi između 30 i 73 godine – prosječna dob 51,33 ± 12,61 godina. Srednje vrijednosti, standardna devijacija (SD), minimalne (min.) i maksimalne (maks.) vrijednosti kliničkih parametara za procijenjene skupine nalaze se u tablici 1.

Prosječni PPD bio je najveći u skupini PI u usporedbi s drugim dvjema skupinama, kao što je prikazano u tablici 1. Taj je parametar bio značajno različit između svih triju skupina. P vrijednosti ispitivanih kliničkih parametara među svim skupinama nalaze se u tablici 2., a svaka je skupina uspoređena s drugima dvjema.

BOP i PI bili su najveći u skupini PM (tablica 1.). Dobivena je značajna razlika u BOP-u između svih skupina, a u parametru PI statistička značajnost izražena je između skupina PI i HI te PM i HI (tablica 2.).

SUP je bio češći u skupini PI nego u skupini PM (tablica 1.), a u skupini HI nije otkriven. Taj parametar nije poka-

Table 1 Mean, standard deviation, minimum and maximum values for examined clinical parameters
Tablica 1. Srednja vrijednost, standardna devijacija, minimalne i maksimalne vrijednosti za ispitivane kliničke parametre

	PPD			BOP			SUP			PI		
	Mean±SD	min	max	Mean±SD	min	max	Mean±SD	min	max	Mean±SD	min	max
PI	4.02±0.77	2.70	5.30	0.67±0.36	0.00	1.00	0.18±0.29	0.00	0.83	0.50±0.36	0.00	1.00
PM	3.39±0.52	3.00	4.50	0.93±0.16	0.50	1.00	0.14±0.15	0.00	0.50	0.65±0.33	0.00	1.00
HI	1.97±0.54	1.30	3.00	0.05±0.11	0.00	0.33	0.00±0.00	0.00	0.00	0.02±0.05	0.00	0.17

Table 2 *P values* for examined clinical parameters among groups. The significance was set at *p<0.05.*
Tablica 2. P-vrijednosti za ispitivane kliničke parametre među skupinama; razina značajnosti postavljena je na p < 0,05

	PPD	BOP	SUP	PI
PI/PM	p=0.036	p=0.049	p=0.806	p=0.354
PI/HI	p<0.001	p=0.001	p=0.031	p=0.001
PM/HI	p<0.001	p<0.001	p=0.005	p<0.001

Table 3 *P values* for the relative level of *Phorphyromonas gingivalis* among groups. The significance was set at *p<0.05.*
Tablica 3. P vrijednosti za relativnu razinu bakterije *Porphyromonas gingivalis* među skupinama; značajnost je postavljena na p < 0,05

	Porphyromonas gingivalis
PI/PM	p=0.049
PI/HI	p=0.012
PM/HI	p=0.470

Table 4 Correlations between the examined clinical peri-implant parameters and relative levels of *Porphyromonas gingivalis*
Tablica 4. Korelacije između ispitivanih kliničkih periimplantantnih parametara i relativnih razina bakterije *Porphyromonas gingivalis*

		PPD	BOP	SUP	PI
Porphyromonas gingivalis	rho	0.229	0.293	0.332	0.131
	p value • p vrijednost	0.223	0.116	0.073	0.491

rho - Spearman's correlation coefficient • Spearmanov koeficijent korelacije

cant difference regarding the appearance between PI and PM groups (Table 2).

The relative level of *Porphyromonas gingivalis* was the highest in the PI group (Figure 3). *P-value* significantly differed among groups PI and HI and PI and PM groups, as it is shown in Table 3, as previously, each group was compared with other two groups. No statistically significant correlations have been noted between the examined clinical periimplant parameters and the relative level of *Porphyromonas gingivalis* (Spearman's rank correlation coefficient, *p>0.05*) *(*Table 4).

Discussion

The findings of the present cross-sectional pilot study indicated that values of recorded clinical parameters were mostly higher in the PI group than in the other two groups. These results are in accordance with available literature data. The study by Ramanauskaite et al indicated that analyzed periimplant clinical parameters such as PPD, BOP, and SUP were associated with the severity of peri-implant diseases. In addition, this study showed higher PPD and BOP in periimplant mucositis and peri-implantitis compared to healthy sites, while SUP was detected in the PI group only (20). More recent research from Monje et al (21), revealed that only PPD significantly differed between PI and PM, while BOP showed a significant difference only when compared between PM and PI to HI groups. PPD exhibited a similar trend in the present and all the previous studies, likewise the conclusion by Monje et al, stating that particularly this parameter might distinguish diagnoses between peri-implant conditions (21). However, it should not be forgotten that the diagnosis of these conditions was obtained by summation of all clinical parameters.

Furthermore, the present study observed higher BOP values in PM than in PI. Additionally, SUP was detected in both PI and PM groups, with greater values in PI, however without significant differences between PI and PM. These findings are not in full accordance with the literature data where BOP exhibited greater values in PI than PM, while SUP was observed only in PI (20). One could hypothesize that the observations noted in the present study might be a consequence of a rather small sample size.

Moreover, this research revealed significantly higher relative levels of *Porphyromonas gingivalis* in PI compared to the other two groups. Similarly, Cortelii et al reported comparable findings where *Porphyromonas gingivalis* and red complex species were more frequent in PI than in HI (22). Additionally, a novel review article also confirmed that Gram-negative anaerobic species were involved in the pathogenesis of the periodontal disease, with *Porphyromonas gingivalis* being one of the bacteria with the highest virulence (23). Furthermore, the present study did not find a statistically significant correlation between recorded clinical parameters and relative levels of *Porphyromonas gingivalis*, with greater mean values in *Porphyromonas gingivalis* levels as the disease progressed, from PM to PI. This result is not in accordance with the available literature, since Rutar et al, stated that PPD was in positive

zao statistički značajnu razliku u izgledu između skupina PI i PM (tablica 2.).

Relativna razina bakterije *Porphyromonas gingivalis* bila je najveća u skupini PI (slika 3.). P-vrijednost značajno se razlikovala među skupinama PI i HI te PI i PM, kao što je prikazano u tablici 3., te je kao i prethodno svaka skupina uspoređivana s drugim dvjema. Nisu zabilježene statistički značajne korelacije između ispitivanih kliničkih periimplantnih parametara i relativne razine *bakterije Porphyromonas gingivalis* (Spearmanov koeficijent korelacije, p > 0,05) (tablica 4.).

Rasprava

Nalazi ovoga presječnoga pilot-istraživanja pokazali su da su vrijednosti zabilježenih kliničkih parametara većinom veće u skupini PI nego u drugim dvjema. Ti su rezultati u skladu s dostupnim podatcima iz literature. U istraživanju koje su proveli Ramanauskaite i suradnici pokazano je da su analizirani klinički parametri, kao što su PPD, BOP i SUP, povezani s ozbiljnošću periimplantatnih bolesti. Osim toga, to je istraživanje pokazalo viši PPD i BOP u slučaju periimplantantnog mukozitisa i periimplantitisa u usporedbi sa zdravim mjestima, a SUP je otkriven samo u skupini PI (20). U novijim istraživanjima Monjea i suradnika (21) otkriveno je da se samo PPD značajno razlikuje između PI-ja i PM-a, dok je BOP pokazao značajnu razliku samo u usporedbi između PM-a i PI-ja prema HI skupinama. PPD je pokazao sličan trend u ovom i svim dosadašnjim istraživanjima, kao i zaključak Monjea i suradnika u kojemu se ističe da se upravo prema tom parametru mogu razlikovati dijagnoze između periimplantantnih stanja (21). No ne treba zaboraviti da se dijagnoza tih stanja dobiva zbrajanjem svih kliničkih parametara.

Nadalje, u ovom istraživanju uočene su veće vrijednosti BOP-a u skupini PM nego u skupini PI. Dodatno, SUP je otkriven u objema skupinama, s višim vrijednostima u PI-ju, ali bez značajnih razlika između PI-ja i PM-a. Ti nalazi nisu potpuno u skladu s podatcima iz literature u kojima je BOP pokazao veće vrijednosti u skupini PI nego PM, a SUP je zabilježen samo u PI-ju (20). Moglo bi se pretpostaviti da bi zapažanja navedena u ovom istraživanju mogla biti posljedica razmjerno male veličine uzorka.

Štoviše, ovo je istraživanje otkrilo značajno više relativne razine bakterije *Porphyromonas gingivalis* u skupini PI u usporedbi s drugima dvjema. Slično tomu, Cortelii i suradnici izvijestili su o usporedivim nalazima u kojima su vrste *Porphyromonas gingivalis* i bakterije crvenoga kompleksa bile češće u PI-ju nego u HI-ju (22). Taj pregledni rad također je potvrdio da su gram-negativne anaerobne vrste bile uključene u patogenezu parodontne bolesti, pri čemu je *Porphyromonas gingivalis* jedna od bakterija s najvećom virulentnošću (23). Nadalje, ovo istraživanje nije pokazalo statistički značajnu korelaciju između zabilježenih kliničkih parametara i relativnih razina bakterije *Porphyromonas gingivalis*, s većim srednjim vrijednostima razina bakterije *Porphyromonas gingivalis* kako je bolest napredovala, od PM-a do PI-ja. Taj rezultat nije u skladu s rezultatima iz dostupne literature jer su Rutar i suradnici izjavili da je PPD u pozitivnoj korelaciji s bro-

correlation with spirochete counts, total anaerobic cultivable bacterial counts, and frequency of *Porphyromonas gingivalis* (24). In addition, the recent report revealed the highest correlation values between PPD and bacterial load parameters of *Porphyromonas gingivalis* (25). However, another novel research also did not find a significant correlation between submucosal microbial dysbiosis and certain clinical parameters (26). As stated before, the present cross-sectional study was pilot research comprising a small number of patients and inserted implants (only 30). Therefore, it has to be emphasized that all the observations made in this study should be interpreted with caution since a larger cohort of patients is needed to confirm or contradict the above-mentioned results.

Conclusions

Within the limitations of this pilot study, it can be concluded that peri-implant diseases, especially peri-implantitis, are characterized by higher relative levels of *Porphyromonas gingivalis*. Clinical parameters in these groups of patients are in correlation with the severity and the stage of the peri-implant disease. If further research continues on a larger patient sample size, the correlation between clinical parameters and relative levels of *Porphyromonas gingivalis* might be detected.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

This research was supported by Grant No. 451-03-68/2022-14/200129 of the Ministry of Education, Science and Technological Development of the Republic of Serbia. The results of the present study were presented at 10th Virtual World Dental Congress, May 4-6th 2022. The authors have declared no conflict of interest.

Author contribution: **N.S:** investigation, data collection, writing - preparation of the original draft; **D.H:** investigation, data collection; **M.J:** methodology, data collection, samples and data analyses; **A.Đ.K:** methodology, visualization, writing – review and editing; **I.M:** conceptualization, methodology, supervision, validation, writing – review and editing.

jem spiroheta, ukupnim brojem anaerobnih bakterija koje se mogu uzgajati i učestalošću bakterije *Porphyromonas gingivalis* (24). Osim toga, nedavno izvješće otkrilo je najviše vrijednosti korelacije između PPD-a i parametara opterećenja bakterijom *Porphyromonas gingivalis* (25). Međutim, u drugom novom istraživanju također nije pokazana značajna korelacija između submukozne mikrobne disbioze i određenih kliničkih parametara (26). Kao što je prije navedeno, ovo presječno istraživanje bilo je pilot-istraživanje koje je obuhvaćalo mali broj pacijenata i ugrađenih implantata (samo 30). Zato se mora istaknuti da sva opažanja u ovom istraživanju treba tumačiti s oprezom jer je potrebna veća skupina pacijenata da bi se potvrdili ili opovrgnuli navedeni rezultati.

Zaključci

Unutar ograničenja ovog pilot-istraživanja može se zaključiti da periimplantatne bolesti, osobito periimplantitis, obilježavaju više relativne razine bakterije *Porphyromonas gingivalis*. Klinički parametri u tim, skupinama pacijenata u korelaciji su s težinom i stadijem periimplantatne bolesti. Ako se daljnja istraživanja nastave na većem uzorku pacijenata, mogla bi se otkriti korelacija između kliničkih parametara i relativnih razina bakterije *Porphyromonas gingivalis*.

Sukob interesa

Autori nisu bili u sukobu interesa.

Zahvala

Ovo istraživanje financirano je u sklopu projekta br. 451-03-68/2022-14/200129 Ministarstva prosvjete, znanosti i tehnološkog razvoja Republike Srbije. Rezultati ovog istraživanja predstavljeni su na 10. Virtualnom svjetskom stomatološkom kongresu od 4. do 6. svibnja 2022.

Doprinos autora: **N. S** – istraživanje, prikupljanje podataka, pisanje, priprema izvornoga nacrta; **D. H** – istraživanje, prikupljanje podataka; **M. J** – metodologija, prikupljanje podataka, uzorci i analiza podataka; **A. Đ. K** – metodologija, vizualizacija, pisanje – pregled i obrada; **I. M** – konceptualizacija, metodologija, nadzor, vrjednovanje, pisanje, recenzija i redakcija.

Sažetak

Svrha istraživanja: Željela se procijeniti potencijalna korelacija između kliničkih periimplantatnih parametara i bakterije *Porphyromonas gingivalis* u različitim stanjima periimplantatnoga tkiva. **Materijal i metode:** Istraživanjem je obuhvaćeno 30 pacijenata Zavoda za parodontologiju i oralnu medicinu podijeljenih u tri jednake skupine definirane prema dijagnozi: periimplantitis (PI), periimplantantni mukozitis (PM) i zdravo periimplantno tkivo (HI). Zabilježeni su klinički parametri kao što su periimplantatna dubina sondiranja (PPD), krvarenje pri sondiranju (BOP), gnojenje pri sondiranju (SUP) i indeks plaka (PI). Prikupljeni su uzorci periimplantatne krevikularne tekućine i analizirane su relativne razine bakterije *Porphyromonas gingivalis* s pomoću PCR-a u stvarnom vremenu (reverzna transkriptaza – lančana reakcija polimeraze u stvarnom vremenu). **Rezultati:** Prosječna dob pacijenata bila je 51,33 ± 12,61 godina. Srednja vrijednost za relativnu razinu bakterije *Porphyromonas ginigvalis* bila je viša u skupini PI (14,80 ±31,51) u usporedbi sa skupinama PM i HI (0,48 ± 1,34, odnosno 0,06 ± 0,12). Taj se parametar značajno razlikovao između PI-ja i HI-ja (p = 0,012) te PI-ja i PM-a (p = 0,049). Prosječni PPD u skupini PI (4,02 mm ± 0,77 mm) također je bio značajno veći u usporedbi s drugim dvjema skupinama – 3,39 mm ± 0,52 mm u PM-u i 1,97 mm ± 0,54 mm u skupini HI. SUP u skupini PI (srednja vrijednost 18 %) bio je češći nego u skupini PM (srednja vrijednost od 14%), a nije otkriven u skupini HI. Korelacija između kliničkih parametara i relativnih razina bakterije *Porphyromonas gingivalis* nije bila značajna. **Zaključak:** Nalazi ovog istraživanja upućuju na više relativne razine bakterije *Porphyromonas gingivalis* u periimplantatnim lezijama, posebice ako je riječ o periimplantitisu.

Zaprimljen: 20. srpnja 2022.
Prihvaćen: 14. listopada 2022.

Adresa za dopisivanje
Ana Đinić Krasavčević
University of Belgrade
School of Dental Medicine
Department of Periodontology and Oral Medicine
Dr. Subotića 4, 11 000 Belgrade, Serbia
ana.djinic@gmail.com

MeSH pojmovi: Porphyromonas gingivalis; periimplantitis; parodontne bolesti; mukozitis
Autorske ključne riječi: periimplantantni mukozitis; zdravo periimplantatno tkivo

References

1. Buser D, Mericske-Stern R, Bernard JP, Behneke A, Behneke N, Hirt HP, et al. Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multicenter study with 2359 implants. Clin Oral Implants Res. 1997 Jun;8(3):161-72.
2. Schiegnitz E, Hill N, Sagheb K, König J, Sagheb K, Al-Nawas B. Short versus standard length implants with sinus floor elevation for the atrophic posterior maxilla. Acta Stomatol Croat. 2022; 56(2): 143.
3. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Clin Periodontol. 2018 Jun;45 Suppl 20:S278-S285.
4. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Clin Periodontol. 2018 Jun;45 Suppl 20:S1-S8.
5. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. J Clin Periodontol. 2018 Jun;45 Suppl 20:S237-S245.
6. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Clin Periodontol. 2018 Jun;45 Suppl 20:S246-S266.
7. Radović M, Gavić L, Jerković D, Željezić D, Puizina J, Srzentić I, et al. Clinical prospective assessment of genotoxic effects of dental implants in gingival epithelial cells. Acta Stomatol Croat. 2022; 56(3): 222-234.
8. Quirynen M, Van Assche N. Microbial changes after full-mouth tooth extraction, followed by 2-stage implant placement. J Clin Periodontol. 2011 Jun; 38(6): 581-589.
9. Fürst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007 Apr; 18(4): 501-508.
10. Heitz-Mayfield LJ, Lang NP. Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. Periodontol 2000. 2010 Jun;53:167-81.
11. Salvi GE, Lang NP. Diagnostic parameters for monitoring peri-implant conditions. Int J Oral Maxillofac Implants. 2004;19.
12. Quirynen M, Vogels R, Pauwels M, Haffajee AD, Socransky SS, Uzel NG, et al. Initial subgingival colonization of 'pristine' pockets. J Dent Res. 2005 Apr;84(4):340-4.
13. Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. Clin Implant Dent Relat Res. 2014 Dec;16(6):783-93.
14. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998 Feb;25(2):134-44.
15. Tzach-Nahman R, Mizraji G, Shapira L, Nussbaum G, Wilensky A. Oral infection with Porphyromonas gingivalis induces peri-implantitis in a murine model: Evaluation of bone loss and the local inflammatory response. J Clin Periodontol. 2017 Jul;44(7):739-748.
16. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011 Nov 17;10(5):497-506.
17. Hajishengallis G. The inflammophilic character of the periodontitis-associated microbiota. Mol Oral Microbiol. 2014 Dec;29(6):248-57.
18. How KY, Song KP, Chan KG. Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. Front Microbiol. 2016 Feb 9;7:53.
19. Araujo MG, Lindhe J. Peri-implant health. J Clin Periodontol. 2018 Jun;45 Suppl 20:S230-S236.
20. Ramanauskaite A, Becker K, Schwarz F. Clinical characteristics of peri-implant mucositis and peri-implantitis. Clin Oral Implants Res. 2018 Jun;29(6):551-556.
21. Monje A, Caballé-Serrano J, Nart J, Peñarrocha D, Wang HL, Rakic M. Diagnostic accuracy of clinical parameters to monitor peri-implant conditions: A matched case-control study. J Periodontol. 2018 Apr;89(4):407-417.
22. Cortelli SC, Cortelli JR, Romeiro RL, Costa FO, Aquino DR, Orzechowski PR, et al. Frequency of periodontal pathogens in equivalent peri-implant and periodontal clinical statuses. Arch Oral Biol. 2013 Jan;58(1):67-74.
23. Luşan SAL, Lucaciu OP, Petrescu NB, Mirică IC, Toc DA, Albu S, et al. The Main Bacterial Communities Identified in the Sites Affected by Periimplantitis: A Systematic Review. Microorganisms.2022 Jun; 10(6): 1232.
24. Rutar A, Lang NP, Buser D, Bürgin W, Mombelli A. Retrospective assessment of clinical and microbiological factors affecting periimplant tissue conditions. Clin Oral Implants Res. 2001 Jun;12(3):189-95.
25. Nastych O, Goncharuk-Khomyn M, Foros A, Cavalcanti A, Yavuz I, Tsaryk, V. Comparison of bacterial load parameters in subgingival plaque during peri-implantitis and periodontitis using the RT-PCR method. Acta Stomatol Croat. 2020 Mar;54(1):32-43.
26. Shi Y, Tong Z, Zhang Y, Si M, He F. Microbial profiles of peri-implant mucositis and peri-implantitis: Submucosal microbial dysbiosis correlates with disease severity. Clin Oral Implants Res. 2022;33(2):172-183.