ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta Stomatol Croat. 2012;46(2):97-104.
IZVORNI ZNANSTVENI RAD
ORIGINAL SCIENTIFIC PAPER

Roberto Legović[1], Andrej Aurer[2]

Evaluacija stanja parodontnih i periimplantantnih tkiva kod pacijenata s dentalnim implantatima

Evaluation of Periodontal and Periimplant Tissues in Patients with Dental Implants

[1] Stomatološka ordinacija Bušćina, Sveta Marija na Krasu
Dental Office Bušćina, Sveta Marija na Krasu
[2] Zavod za parodontologiju Stomatološkog fakulteta Sveučilišta u Zagrebu
Department of Periodontology School of Dental Medicine University of Zagreb

Sažetak
Svrha: Željelo se usporediti stanje periimplantatnih i parodontnih tkiva parodontnim indeksima (API, BOP, PD) i multipleksnom PCR-metodom. **Ispitanici i postupci:** U istraživanju je sudjelovalo 25 pacijenata – 12 muškaraca i 13 žena u dobi od 32 do 69 godina. Svi su imali ugrađene oseointegrirane dentalne implantate u rasponu od deset mjeseci do pet godina. Kod 16 ispitanika obavljena je PCR-analiza briseva oko prirodnih zuba i implantata na prisutnost pet parodontnih patogena – Aa, Pg, Pi, Tf, Td. Prikupljeni podaci statistički su obrađeni programom SPSS for Windows 16,0. **Rezultati:** Ovo istraživanje pokazalo je da nema veće razlike u parodontološkim parametrima oko zuba i implantata. Mikrobiološkom analizom pronađeno je u prosjeku 3,56 bakterijskih vrsta po pacijentu oko zuba i 3,5 bakterijskih vrsta oko implantata. PCR-analizom Aa je pronađen kod 68,8 posto sudionika oko zuba i kod njih 75 posto oko implantata. Pg je nađen kod 82 posto ispitanika oko zuba i 87 posto sudionika oko implantata. Pi je pronađen kod 32 posto ispitanika oko zuba i 25 posto njih oko implantata. Tf je bio pronađen kod oko 88 posto ispitanika oko zuba i 94 posto sudionika oko implantata, a Td kod 87 posto oko zuba i 68 posto oko implantata. Nađena je i velika korelacija vremena proteklog od implantacije i prisutnosti bakterije Td, umjerena korelacija za Aa i slaba korelacija za Pi i Tf. Pronađena je izrazito jaka povezanost te iste bakterije oko zuba i implantata za sve ispitivane bakterije ($p<0,05$). **Zaključak:** Kombinacija visokih vrijednosti plaka i krvarenja, uz prisutnost parodontnih patogena, čini se, može prouzročiti periimplantitis.

Zaprimljen: 28. ožujka 2012.
Prihvaćen: 16. svibnja 2012.

Adresa za dopisivanje
Doc.dr.sc. Andrej Aurer
Sveučilište u Zagrebu
Stomatološki fakultet
Zavod za parodontologiju
Gundulićeva 5
Tel: + 385 1 4802 155
Fax: + 385 1 4802 159
aurer@sfzg.hr

Ključne riječi
periimplantitis; zubni usadci; zubni plak; PCR

Uvod

Između parodontitisa i periimplantitisa postoje sličnosti, ali i jasne razlike uvjetovane razlikama u anatomskoj građi tkiva gingive i mukoze oko implantata, što rezultira razlikom u reakciji obrambenog sustava i u progresiji bolesti (1). Prema Albrektssonu i Isidoru (2), periimplantitis je definiran kao upalni proces koji zahvaća tkiva oko oseointegriranog implantata u funkciji i rezultira gubitkom potporne kosti. Njegov predstadij je periimplantatni mukozitis, reverzibilna upalna reakcija na sluznici uz dentalni implantat (3, 4). Upalne promjene oko implantata nisu ograničene na vezivno tkivo, nego zahvaćaju i alveolnu kost (5) te u slučaju napredovanja mogu rezultirati gubitkom implantata.

Patogeni mikroorganizmi u gingivnom plaku, posebice *Porphyromonas gingivalis* (Pg), *Tannerela forsythia* (Tf) te *Actinobacillus actinomycetemcommitans* (Aa) (6 - 9), povezani su s težim oblicima parodontitisa, te su jedan od najčešćih uzroka gubitka zuba (10 -12). Ono što povezuje sva istraživanja o parodontitisu česti su gram-negativni patogeni anaerobi ko-

Introduction

Periodontitis and periimplantitis exhibit similarities, but also clear differences determined by different anatomical structure of the gingiva around teeth and the mucosa around the implants, which leads to different reactions of the host defense and the progression of the disease (1). According to Albrektsson and Isidor (2), periimplantitis is an inflammatory process which involves the tissues around osseointegrated implants during the function, resulting in the loss of the supportive bone. Periimplantitis is prequeled by periimplant mucositis, a reversible inflammatory reaction on the mucosa surrounding the dental implant (3, 4). Inflammatory changes around implants are not restricted to connective tissue, but also affect the alveolar bone (5) and if they progress can lead to implant loss.

The presence of the periodontopathogenic microorganisms in gingival plaque, especially *Porphyromonas gingivalis* (Pg), *Tannerela forsythia* (Tf) and *Actinobacillus actinomycetemcommitans* (Aa) (6-9), is related to severe periodontitis cases

ji se nalaze u dubljim slojevima periimplantatnih lezija (13 - 15). Svi navedeni patogeni primarno su vezani za prirodne zube i mogu se naseliti u usatke tijekom šest mjeseci nakon što se postave u bezube dijelove čeljusti (16).

Zato je potrebno prije implantološko-protetičke terapije sanirati parodont. Sanirani parodont (17, 18) i higijenski kvalitetna okolina usne šupljine uvjet je za uspješno prihvaćanje dobro planirane terapije djelomične i potpune bezubosti dentalnim implantatima. Kako je periimplantitis teška parodontna bolest koja može završiti progresivnom difuznom destrukcijom potporne kosti i okolnih tkiva, sigurno je vrlo važno na vrijeme identificirati lokalne parametre koji u većoj mjeri utječu na inicijaciju, odnosno na progresiju bolesti.

Metoda lančane reakcije polimeraze (PCR) ima važno mjesto u parodontnoj dijagnostici. Ta laboratorijska metoda oponaša postupak prepisivanja DNK (replikaciju) koji se događa u svakoj živoj stanici. U kliničkoj dijagnostici dovoljan je mali uzorak genetskog materijala (DNK) za otkrivanje pojedinog patogena ili promjene gena vlastitih stanica. Tim postupkom znatno je poboljšana dijagnostika infekcije. Multipleksni PCR odlikuje se mogućnošću istodobnog otkrivanja više patogenih mikroorganizama u uzorcima subgingivnog plaka, pa se tako mogu istodobno detektirati tri i više različitih parodontnih patogena (19).

U ovom radu usporedit će se stanje periimplantatnih i parodontnih tkiva kod pacijenata liječenih dentalnim implantatima, te nastojati uočiti sličnosti i razlike u ponašanju tih tkiva. Zbog toga su se mjerili parodontni indeksi koji objektivno i reproducibilno opisuju zdravlje i stupanj zahvaćenosti parodontnih struktura bolešću prirodnih zuba i dentalnih implantata (20, 21). PCR-metodom željeli smo dokazati prisutnost parodontnih patogena u dubljim strukturama tkiva oko zuba i implantata.

Ispitanici i postupci

Istraživanjem je bilo obuhvaćeno 25 pacijenata koji su pregledani u privatnoj stomatološkoj ordinaciji. Sudjelovalo je 12 žena i 13 muškaraca u dobi od 32 do 69 godina. Svi su imali ugrađene oseointegrirane dentalne implantate od deset mjeseci do pet godina – u prosjeku oko dva implantata po pacijentu. Svim sudionicima izmjereni su parodontni indeksi, a kod njih 16 primijenjena je i PCR-analiza briseva oko prirodnih zuba i implantata. U skupini ispitanika nije bilo nikoga s implantatima zbog potpune bezubosti, nego su sudionici morali imati najmanje tri prirodna zuba u svakoj čeljusti zbog usporedbe parodontnog statusa. Implantati su bili od titana, cilindričnog oblika s navojima (Astra Techz, Goteborg Švedska). Pacijenti posljednjih šest mjeseci nisu bili parodontološki liječeni, nisu dobivali antibiotike i nisu bolovali od sustavnih bolesti – dijabetesa, kardiovaskularnih bolesti, neutropenije i dr. zbog moguće uzročne veze tih bolesti i bolesti parodonta (22).

Svi ispitanici potpisali su suglasnost o sudjelovanju (informirani pristanak). Pregled na stomatološkom stolcu uklju-

and is one of the most common reasons for tooth loss (10-12). The fact that connects all investigations in the nature of periodontitis is frequent finding of gram negative anaerobic pathogens located in the deeper portions of periimplant lesions (13-15). All mentioned periodontal pathogens are found primarily on the natural teeth, but can also colonize implants during the 6 months following the implant placement (16).

Before starting the prosthetic therapy in implants, it is necessary to ensure healthy periodontal conditions. Periodontal health (17, 18) and excellent plaque control are conditions for the success of a well planned treatment of partial or total tooth loss with dental implants. Since periimplantitis is a serious condition that can lead to progressive destruction of the supporting alveolar bone and adjacent tissues, it is of general interest to identify the local parameters which significantly influence the initiation and progression of this disease.

Polymerase Chain Reaction (PCR) method holds an important place in periodontal microbiological diagnosis. This laboratory method mimics the translation of the DNA (replication) which takes place in every living cell. For the clinical diagnostics, a very small sample of the genetic material (DNA) suffices for the detection of the specific pathogen. This procedure has substantially advanced the bacterial diagnosis of the infection. Multiplex PCR allows for the simultaneous detection of three or more pathogenic microorganisms in the subgingival plaque samples (19).

This investigation compared the status of periimplant and periodontal tissues in the patients treated with dental implants, and observed the similarities or differences in the behavior of these tissues. Clinical periodontal indices were used for this purpose, which allow objective and reproducible measurement of health and the degree of the involvement of periodontal and periimplant structures in the inflammatory processes (20, 21). PCR method was used to detect the presence of the periodontal pathogens in deeper parts of the tissues surrounding teeth and implants.

Material and methods

This study included 25 subjects examined in private practice. There were 12 males and 13 females, between 32 and 69 years of age. All subjects were treated with osseointegrated dental implants, on average 2 implants per patient. The time elapsed from the implant placement was between 10 months and 5 years. Periodontal indices were recorded in all patients, and 16 subjects were microbiologically tested, using semiquantitative multiplex PCR with the samples taken from natural teeth and implants. Each patient had to have at least 3 natural teeth per jaw, and the implants were cylindrical screwed-type titanium implants (Astra Tech, Sweden, Goteborg). All patients who had periodontal treatment or antibiotic treatment in the last 6 months were excluded, as were the patients who were suffering from systemic diseases such as diabetes, cardiovascular disorders, etc., due to the possible interrelationship (22).

All patients gave their informed written consent for the participation in this study. The clinical and microbiological data were recorded. Clinical examination included measure-

čivao je određivanje sljedećih parodontalnih indeksa: API – aproksimalni indeks plaka (23), BOP – krvarenje pri sondiranju (24, 25), PD – dubinu sondiranja i R– recesiju gingive. Endoralnom rentgenskom snimkom procjenjivali smo odnos kosti i oseointegriranog implantata, tijek *limbus alveolaris*, te vertikalnu ili horizontalnu resorpciju kosti.

Metodom lančane reakcije polimeraze (PCR) dokazivali smo parodontne patogene: *Actinobacillus (Aggregatibacter) actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf) i *Treponema denticola* (Td). Prije toga uzeti su uzorci i izdvojen DNK (61). Korišten je molekularno-genetski test za identifikaciju pet parodontopatogenih bakterija *micro-IDent,* molekularni test tvrtke Hain Lifescience GmbH, Nehren, Njemačka. U izvođenju testova strogo su se poštovale upute proizvođača testa koje su sastavni dio kompleta za testiranje (www.hain-lifescience.de/pdf/protokoll). Analiza je obavljena u poliklinici *Virogena*, u Zagrebu, u Hrvatskoj. Od instrumenata korištena je parodontna sonda (PCP-11, Hu-Friedy, Chicago, Illinois, SAD) i stomatološko zrcalo paper-point veličine 25 ili 30 (Paper Points, Roeko, Langenau, Njemačka).

Prikupljeni podaci statistički su obrađeni programom SPSS for Windows 16,0. Normalnost raspodjele podataka testirana je Smirnov-Kolmogorovljevim testom. Usporedba nezavisnih uzoraka obavljena je Studentovim t-testom. Za zavisne uzorke korelacija je određena Pearssonovim testom korelacije.

Rezultati

U istraživanju je sudjelovalo 25 ispitanika s ugrađenim dentalnim implantatima. Među njima je bilo 48 posto žena i 52 posto muškaraca. Mikrobiološka analiza patogenih bakterija provedena je na 64 posto ukupnog uzorka. U prosjeku je svakom pacijentu bilo ugrađeno 1,96 ± 1,06 implantata, a vrijeme proteklo od implantacije u prosjeku je iznosilo 24,5 ± 12,27 mjeseci. Prosječna dob pacijenata bila je 51,6 ± 10,33 godine.

Dubina džepova određivala se za pojedine skupine zuba – prednji zubi, pretkutnjaci i kutnjaci. Srednja vrijednost dubine sondiranja iznosila je 1,66 ± 0,58 milimetara za zube, a za implantate 1,47 ± 0,78. Vrijednosti PD-a i R-a po pojedinim skupinama zuba nalaze se u tablici 1. Srednja vrijednost za recesije kod prirodnih zuba bila je 0,25 ± 0,25 milimetara, a za implantate 0,12 ± 0,7 milimetara, što je bilo statistički značajno (p=0,045). Nije pronađena statistički značajna razlika u dubini sondiranja između zuba i implantata.

Nije bilo razlike u distribuciji podataka prema spolu. Studentovim t-testom za zavisne varijable pokazano je da nement of the following periodontal indices: API- Aproximal Plaque Index (23), BOP- Bleeding on Probing (24, 25), PD- Probing Depth, R- Gingival Recession. Intraoral radiographs were taken for assessment of the bone level surrounding the osseointegrated implant, and horizontal or vertical bone resorption.

Polymerase chain reaction (PCR) method was used to detect the presence of periodontal pathogens: *Actinobacillus (Aggregatibacter) actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf) and *Treponema denticola* (Td). The samples were taken using sterile paper points inserted in the 5 deepest pockets around teeth or implants pooled separately, for each patient. DNA was extracted prior to the semiquantitative PCR (61). Molecular genetic test for the identification of the 5 periodontal pathogens 'micro-IDent', Hain Lifescience GmbH, Nehren, Germany, was used. Tests were performed strictly according to the instructions for use by the manufacturer, which were integral part of the testing kit (www.hain-lifescience.de/pdf/protokoll). Laboratory analysis was done in the Polyclinic "Virogena", Zagreb, Croatia. The clinical indices were taken using periodontal probe (PCP-11, Hu-Friedy, Chicago, Illinois, USA), and dental mirror. Samples were taken with paper points size 25 and 30 (Paper points Isocolor, Roeko, Langenau Germany).

Collected data were statistically evaluated using SPSS for Windows 16.0 software. The normality of distribution was tested using Smirnov-Kolmogorov test. Independent samples were tested using Student t-test. Pearsson correlation test was used to test the correlations between dependent variables.

Results

Investigation included 25 patients treated for tooth loss with dental implants; of these 48 % were female and 52% male. Microbiological detection of the pathogenic bacteria was undertaken on 64% of the samples. An average of 1.96 ± 1.06 implants per patient were found, and mean time elapsed from the implant placement was 24.5 ± 12.27 months. Mean patient age was 51.6 ± 10.33 years.

The probing depth and recession mean values were analyzed separately for anterior teeth, premolars and molars. Mean probing depths were 1.66 ± 0.58 mm for teeth and 1.47 ± 0.78 mm for implants. The values for PD and R distributed between teeth groups are shown in Table 1. Mean gingival recession for the natural teeth was 0.25 ± 0.25 mm, and 0.12 ± 0.27 mm for the implants, which was statistically significant (p=0.045). No significant difference was found in probing depths between natural teeth and implants.

No gender-specific differences in the distribution of collected data were observed. Paired samples t-test revealed no statistically significant differences between teeth and im-

Tablica 1. Srednje vrijednosti dubine sondiranja i gingivne recesije za zube i implantate.
Table 1 Mean probing depth and gingival recession values for teeth and implants

	Prednji zubi • Anterior teeth	Premolari • Premolars	Molari • Molars	Zubi • Teeth	Implantati • Implants
PD	1.67 ± 0.45	1.73 ± 0.56	1.58 ± 0.85	1.66 ± 0.58	1.47 ± 0.78
R	0.33 ± 0.19	0.22 ± 0.17	0.17 ± 0.15	0.25 ± 0.25	0.12 ± 0.27

ma statistički značajne razlike za PI i BOP, iako je uočena razlika za recesije od oko 0,1 milimetar.

Mikrobiološkom analizom u prosjeku je svakom pacijentu pronađeno 3,56 bakterijskih vrsta oko zuba i 3,5 bakterijskih vrsta oko implantata. Ta razlika nije statistički značajna. Prevalencija parodontnih patogena oko implantata i zuba prikazana je u tablici 2. Najčešća bakterija oko zuba i implantata bila je Tf, a najrjeđa Pi. Razlika u detekciji pet parodontnih patogena oko zuba i implantata nije bila značajna. Ispitivanjem povezanosti vremena proteklog od implantacije i prisutnosti bakterija Pearsonovim testom korelacije uočena je značajna korelacija za Td (r=0,547, p=0,014) i Aa (r=0,445, p=0,042), a vrijednosti za Pi i Tf nisu bile značajne.

Prisutnost bakterija detektirana je kao slaba, umjerena i jaka. Prevalencija jake prisutnosti rjeđe je opažena u skupini implantata. Primjer distribucije prikazan je na slikama 1a i b.

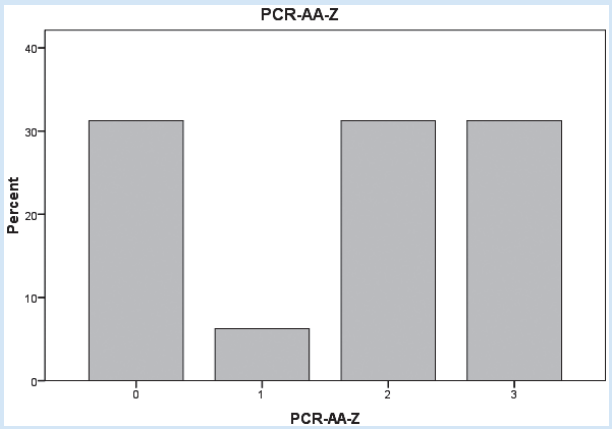
plants in PI, BOP, although difference of approximately 0.1 mm was observed for recession.

Microbiological analysis detected an average of 3.56 bacterial species around teeth and 3.5 bacterial species around implants, which was not significantly different. Prevalence of periodontal pathogens around teeth and implants is shown in Table 2. The most frequently detected bacteria around teeth, as well as implants were Tf, while the least frequently detected periodontal pathogen was Pi. The differences in the detection of the 5 periodontal pathogens around teeth and implants were not significant. The correlation of time since implant placement and the presence of bacteria tested with Pearson correlation test revealed significant correlations for Td (r=0.547, p=0.014) and Aa (r=0.445, p=0.042), while the values for Pi and Tf were not significant.

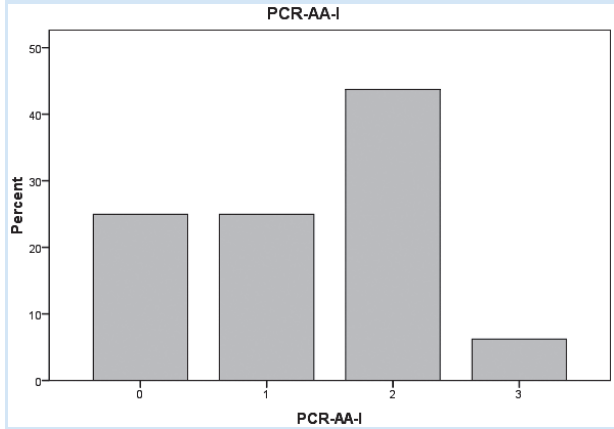
The presence of bacteria was detected as weak, moderate or strong. The prevalence of strong presence as detected by PCR was less frequently observed in the implants group. An example of distribution is shown in Images 1a and b.

Tablica 2. Prevalencija identificiranih patogena oko zuba i implantata
Table 2 Prevalence of identified pathogens in teeth and implants

	Aa	Pg	Tf	Td	Pi
Zubi • Teeth	68.8%	82%	88%	87%	32%
Implantati • Implants	75%	87%	94%	68%	25%



Slika 1a. Semikvantitativna PCR-analiza prisutnosti Aa oko zuba (0 = nema bakterije; 1 = slaba prisutnost; 2 = umjerena prisutnost; 3 = jaka prisutnost)
Image 1a Semiquantitative PCR analysis of Aa on natural teeth. (0 = no bacteria, 1 = weak presence, 2 = moderate presence, 3 = strong presence).



Slika 1b. Semikvantitativna PCR-analiza prisutnosti Aa na površini implantata (0 = nema bakterije; 1 = slaba prisutnost; 2 = umjerena prisutnost; 3 = jaka prisutnost)
Image 1b Semiquantitative PCR analysis of Aa on implant surfaces. (0 = no bacteria, 1 = weak presence, 2 = moderate presence, 3 = strong presence).

Analizom odnosa API-a, PD-a i BOP-a između zuba i implantata po parovima pronađena je snažna povezanost između indeksa plaka zuba i implantata – p<0,01. Također je pronađena jaka povezanost između BOP-a zuba i BOP-a implantata (p<0,01) i jaka povezanost BOP-a zuba i dubine sondiranja implantata (p<0,01).

Pearsonov test korelacije proveden je za krvarenje pri sondiranju i nalaz PCR-analize parodontnih patogena. Pronađena je velika pozitivna korelacija između krvarenja pri sondiranju i nalaza Pi-a (r=0,427, p=0,049) te Tf-a (r=0,496,

Pairwise analysis of API, PD and BOP values of natural teeth and implants revealed a strong correlation between the plaque index values for teeth and implants (p<0.01). Strong correlation between BOP for teeth and implants was found (p<0.01), as well as strong correlation between BOP values on teeth and PD around implants (p<0.01).

Pearsson correlations were calculated for BOP and presence of periodontal pathogens as detected with PCR. Strong correlation was observed between positive BOP and the presence of Pi (r=0.427, p=0.049) and Tf (r=0.496, p=0.025).

p=0,025). Analizom korelacije između dubine sondiranja i PCR-a nije pronađena značajna korelacija. Analizom povezanosti prisutnosti istog parodontnog patogena oko zuba i implantata pronađena je izrazito velika povezanost za sve ispitivane bakterije (tablica 3).

No correlation was revealed between probing depth values around teeth or implants and the presence of periodontal pathogens. The presence of the same periodontal pathogen around natural teeth and implants was significantly correlated for all of the tested bacteria (Table 3).

Tablica 3. Istodobna prisutnost iste bakterijske vrste oko zuba i implantata
Table 3 The simultaneous presence of the same bacterial species around teeth and implants

Bakterijska vrsta • Bacterial species	Aa	Pg	Pi	Tf	Td
Pearsonova korelacija • Pearson correlation	0.777	0.514	0.497	0.555	0.579
p-vrijednost • p value	p<0.001	p=0.021	p=0.025	p=0.013	p=0.009

Samo za Aa, prisutnost na zubima znatno je povećala mogućnost za prisutnost Pi-a (r=0,542, p=0,015) i Tf-a (r=0,478, p=0,031), a za Td (r=0,592, p=0,008) korelacija je bila velika. Nisu pronađene korelacije za ostale testirane bakterije. Kod implantata je prisutnost Td-a značajno vezana za detekciju Aa (r=0,659, p=0,003) i Tf-a (r=0,459, p=0,037).

Analizom intraoralnih rentgenskih slika svih 25 pacijenata, kod ukupno 49 implantata kost je u većini slučajeva bila pravilno formirana bez vidljivih znakova resorpcije. U četiri slučaja uočena je resorpcija kosti u rasponu od 1 do 4 milimetra, što iznosi osam posto od ukupnog ispitivanog uzorka. U ta četiri slučaja vrijeme proteklo od implantacije iznosilo je od 30 do 60 mjeseci, a nađeno je statistički značajno više plaka i krvarenja nego kod ostalih (p<0,05). Kod troje pacijenata s periimplantitisom pronađene su visoke razine svih pet patogenih bakterija, a samo kod jednoga je PCR-analiza pokazala niže razine.

Only for Aa, the presence on natural teeth significantly increased the chances for the presence of Pi (r=0.542, p=0.015) and Tf (r=0.478, p=0.031), while for Td (r=0.592, p=0.008), the correlation was very strong. No such correlations were observed for other bacterial species tested. In implants, the presence of Td was significantly linked to detection of Aa (r=0.659, p=0.003) and Tf (r=0.459, p=0.037).

Intraoral x-rays were analyzed for all 25 patients, of the total of 49 implants. In most of the cases, bone was regularly formed around implants, without visible signs of bone resorption. In 4 cases bone resorption was observed ranging from 1-4 mm, amounting to 8% of the total number of implants. In these 4 cases, time elapsed since implant placement was 30 to 60 months, and these patients had significantly more plaque and bleeding than others (p<0.05). Of these, 3 had very high, and one harbored low levels of all 5 tested periodontal pathogens.

Rasprava

Uspoređujući stanje periimplantatnih i parodontnih tkiva parodontnim indeksima i PCR-mikrobiološkom metodom uočili smo da nije bilo značajnih razlika između njih u pregledanoj populaciji ispitanika. To upućuje na to da kod parodontno zdravih ispitanika s dobrom oralnom higijenom, slični uvjeti mogu postojati i u periimplantatnim tkivima. Dokazano je da bakterije vrlo brzo koloniziraju implantate – od šest do deset mjeseci nakon usađivanja. To potvrđuje studija iz 2004. godine u kojoj su Takanashi i suradnici dokazali da bakterijska kolonizacija Pg-a i Pi-a nastaje rano nakon što je implantat eksponiran intraoralnoj flori kada bakterije prelaze s prirodnih zuba na površinu implantata, i to u tri od četiri slučaja (75 %). Koristili su se 16sRNA specifičnim primerom (20).

Dobiveni rezultati pokazuju da se učestalost parodontnih patogena slična nađenoj na površinama prirodnih zuba može očekivati i na površinama implantata neko vrijeme nakon izlaganja usadaka okolišu u oralnoj šupljini. Podaci također upućuju na to da vrijeme proteklo od implantacije značajno korelira s prisutnošću Aa i Td-a, dok je za Pi i Tf relativno blizu značajnosti. Prisutnost Aa na prirodnim zubima povećava vjerojatnost prisutnosti triju ostalih ispitivanih bakterijskih vrsta na implantatima. Nejasno je povećava li to opasnost od periimplantitisa oko implantata kolonizira-

Discussion

Comparison of the periimplant and periodontal tissues using clinical periodontal indices and microbiological sampling revealed no significant differences in our subjects. This indicates that in periodontally healthy patients with good oral hygiene, favorable conditions could prevail in periimplant tissues as well. It has been shown that bacteria colonize the implants rather fast, in first 6 to 10 months after the implant placement. In a study from 2004, Takanashi et al. showed that bacterial colonization of Pg and Pi takes place early after the implant is exposed to the intraoral microflora whereas bacteria transfer from natural teeth to the implant surface in 3 out of 4 cases (75%). They used 16sRNA specific primer (20).

Our data show that the same incidence of periodontal pathogens present on the surfaces of natural teeth can be expected on the implant surface, sometime after exposing the implants to the milieu of oral cavity. Also, our results show that time since implant placement correlates significantly with the presence of Aa and Td, while for Pi and Tf almost reached significance. The presence of Aa on natural teeth, seems to increase the chances for presence of other three tested bacterial species on implants. It is unclear if this also increases the risk of developing inflammatory periimplant disease around implants colonized with periodontal patho-

nih parodontnim patogenima. Kod pacijenata koji su imali Td na površinama implantata nađena je korelacija s prisutnošću Tf-a i jaka korelacija s Aa. Dobro je poznato da Tf i Td pripadaju izrazito patogenom, tzv. crvenom bakterijskom kompleksu. Trend da je količina nađenih bakterija uglavnom viša za prirodne zube, negoli za implantate mogao bi se objasniti duljom izloženošću zuba oralnom okolišu i mikroflori u odnosu na implantate.

Poznato je da nepotrebno ponovno sondiranje oko implantata može rezultirati oštećenjem pričvrstka i nepovoljnom remodelacijom kosti. Opažena je korelacija između pozitivnih BOP-vrijednosti na prirodnim zubima i dubljih PD-vrijednosti. Daljnje istraživanje potrebno je kako bi se razjasnilo postoji li, i u kojoj mjeri, takva povezanost jer bi mogla biti korisna za kliničko odlučivanje kad postoji dilema je li potrebno sondirati oko implantata.

Sumida i suradnici su PCR-metodom određivali rizik od nastanka periimplantitisa kolonizacijom patogenih bakterija Pg, Pi, Aa, Tf i Td, uspoređujući briseve iz parodontnih džepova s onima oko oseointegriranih implantata (21) na uzorku od 15 pacijenata. Naime, kod Sumide se pokazalo da je kolonizacija Pg-a i Aa između parodontnih džepova i sulkusa implantata bila statistički bez značajnije razlike ($p \leq 0,01$). Učestalost pojedinih bakterija iznosila je: Pg – 80 posto, Pi – 53,3 posto, Aa – 46,7 postpo, Tf – 60 posto i Td – 40 posto. U našem istraživanju učestalost pojedinih parodontnih patogena oko implantata pacijenata iznosi: Aa – 75 posto, Pg – 87 posto, Tf – 94 posto i Td 68 – posto. Možemo zaključiti da su rezultati istraživanja slični. Češća prisutnost nekih patogena, kao Td-a u našem istraživanju, može se pripisati sofisticiranijoj metodi PCR-analize kod koje su primeri očito specifičniji nego u navedenim studijama (Mikrodent analiza tvrtke Hain, Njemačka). Sumida zaključuje da se samo uklanjanjem navedenih patogena može očekivati da će se smanjiti rizik od periimplantitisa. Ti nalazi potvrđuju pretpostavku da parodontni patogeni mogu kolonizirati površinu implantata jednako uspješno kao i prirodne zube, što zahtijeva oprez pri terapiji implantatima kod pacijenata s parodontitisom jer upalna parodontna bolest i parodontni patogeni moraju biti potpuno pod kontrolom prije početka terapije implantatima. Ako pacijenti imaju očuvan, zdrav parodont, redovito održavanje čini se adekvatnom terapijskom opcijom jer još uvijek postoji opasnost od razvoja periimplantatne upale oko implantata.

Serino i Strom objavili su zanimljivo istraživanje o periimplantitisu kod djelomice bezubih pacijenata (26). Oni su na čak 53 posto implantata kod 23 pacijenta našli džepove dubine ≥ 6 milimetara i ustanovili su na samo šest posto zuba dubinu džepa od ≥ 6 mm, što je znatno drugačije od naših rezultata – naš je postotak mnogo manji i iznosi osam posto, što je ustanovljeno PD-sondiranjem i rentgenskim nalazima. Serino i Strom navode da je, kad je riječ o održavanju oralne higijene, nemogućnost pristupa tim implantatima bila važan razlog za nastanak dubokih džepova, što nije slučaj u našem istraživanju. U svakom slučaju redovita oralna higijena i kontrola znatno povećavaju trajnost i dugovječnost implantata.

Abreu i suradnici (27) uspoređivali su stanje prirodnih zuba i implantata kod 41 osobe koristeći se sličnim parame-

gens. In patients harboring Td on implant surfaces, correlation with Tf and strong correlation with Aa was shown. It is well known that Tf and Td are members of the 'red bacterial complex', which is extremely pathogenic. The trend that the amount of detected bacteria is generally higher for the natural teeth group might be attributed to much longer exposure to the oral milieu and microflora in relation to implants.

It is known that unnecessary probing around implants can lead to attachment damage and unfavorable bone remodeling. We observed a correlation between positive BOP values on natural teeth and deeper PD values. Further research is needed to elucidate to which extent such potential relationship exists, since it could be a useful guideline for clinical decision-making when faced with the dilemma whether to probe in patients treated with dental implants.

Sumida et al. used PCR to calculate the risk of periimplantitis from colonization of the pathogenic bacteria Pg, Pi, Aa, Tf and Td, comparing the samples from periodontal pockets with those around osseointegrated implants in 15 patients (21). They showed that there was no statistically significant difference in the colonization rate of Pg and Aa between periodontal pockets and implant sulcus ($p \leq 0.01$). The incidence of single bacterial species was: Pg 80%, Pi 53.3%, Aa 46.7%, Tf 60% and Td 40%. We found the following prevalence of periodontal pathogens around implants: Aa 75%, Pg 87%, Tf 94% and Td 68% of the patients. We can conclude that the results are similar. A more frequent finding of some pathogens, such as Td in our study can be attributed to the more sophisticated PCR method, with more specific primers than used in their study. Sumida concludes that only by removing the pathogens the risk of the periimplantitis can be lowered. These findings support the notion that periodontal pathogens can colonize implant surfaces as readily as natural teeth, indicating caution when treating periodontitis patients with dental implants, since the inflammatory periodontal disease and periodontopathogens have to be fully controlled before implant treatment is initiated.

In patients with uncompromised, healthy periodontium, regular recall seems a reasonable treatment option, since the implants are still at risk of developing periimplant inflammation that could lead to therapy failure.

Serino and Strom published an interesting investigation on periimplantitis in partially edentulous patients (26). They found pockets deeper than 6 mm on 53% of the implants and only 6% of the teeth examined in 23 patients, which is considerably different from our findings of 8% as measured by probing and x-ray. They claimed that the inability to access these implants for proper plaque removal constituted an important reason for such deep pockets, which was not the case in our investigation. However, proper oral hygiene and recall prolong the implant survival.

Abreu et al. (27) compared the conditions around natural teeth and implants in 41 persons using similar clinical parameters, x-ray evaluation of the bone level and PD. Slightly higher values were found around implants in comparison to natural teeth, but these were not statistically significant. Implants showed an average bone loss of 0.77 mm per year, or in percentages 17.11% of bone loss around implants. They

trima, i to rentgenskom evaluacijom razine kosti i PD-om. Oko implantata pronađene su malo veće vrijednosti nego oko prirodnih zuba, no nisu značajne. Implantati pokazuju prosječan gubitak kosti od 0,77 milimetara na godinu ili u postocima 17,11 posto je gubitak kosti oko implantata. Destrukcija tkiva oko implantata veća je, smatraju oni, i to zbog remodelacije tkiva. Renvert i njegovi kolege (28) periimplantitis su našli kod 14 posto ispitanika, što je slično našem postotku od 8 posto. Cheung i njegov tim (29) analiziraju stanje dentalnih implantata nakon rekonstruktivnih zahvata s autogenim koštanim transplantatima ilijačne kosti. Pet godina nakon implantacije trajanje implantata bilo je veće od 90 posto. Zaključili su da se, ako se zahvati pomno planiraju i stručno vode, uz visok stupanj oralne higijene postižu vrlo dobri rezultati, što potvrđuju i naši rezultati.

Zaključak

U istraživanju na ispitivanom uzorku nije bilo statistički značajnih razlika u parodontološkim parametrima oko zuba i implantata. Parodontni patogeni koloniziraju površinu implantata nekoliko mjeseci nakon ugradnje. Oprez je potreban kod pacijenata s neliječenim upalnim parodontnim bolestima. Čini se da problemi nastaju ako pacijenti istodobno imaju visoke vrijednosti indeksa plaka i upale te parodontne patogene. Ta kombinacija je pogodna za razvoj periimplantitisa. U periimplantatnom tkivu u slučaju nedovoljne oralne higijene mogu nastati difuzna upala i destrukcija kosti. Pravodobno dijagnosticiranje problema produžit će trajnost implantata, a mnoge recentne studije potvrđuju da supresijom najvažnijih patogenih bakterija stvaramo zdravu okolinu u kojoj implantati mogu trajati i biti važni u oralnoj rehabilitaciji (30).

Izjava

Autori negiraju bilo koji sukob interesa.

agreed that tissue destruction around implants is higher due to tissue remodeling. Renvert et al. (28) found periimplantistis in 14% of the subjects, which is similar to our findings. Cheung et al. (29) analyzed the state of dental implants after reconstructive procedures using autogenic bone grafts from the iliac region. Five years after the surgery, implant survival was over 90%. They concluded that if the treatment is carefully planned and skillfully performed, and the level of oral hygiene is very high, very good results can be achieved, which is confirmed by our results.

Conclusion

This study found no statistically significant differences between periodontal parameters around natural teeth and implants in the investigated sample. Periodontal pathogens colonize the surface of implants several months after the insertion. Caution should be exercised in patients with untreated inflammatory periodontal disease. It seems that problems arise more often in patients with high plaque and inflammation levels and simultaneously harbor periodontal pathogens. This combination predisposes to periimplantitis. Periimplant tissues in cases of inadequate level of oral hygiene develop a diffuse inflammation with bone destruction. Timely diagnosis can lengthen the implant survival, and many recent studies confirm that suppression of the main periodontal pathogens creates a healthy environment in which implants can last and prove important for the oral rehabilitation procedures (30).

Transparency declaration

The authors deny any conflicts of interest.

Abstract

Aim: Comparison of periodontal and periimplant tissues in health, using clinical periodontal indices and semi-quantitative multiplex PCR method for detection of periodontal pathogens. **Material and methods:** Investigation included 25 subjects, 12 males and 13 females aged between 32 and 69 years. All subjects were treated with osseointegrated dental implants. Microbiological samples taken from pockets around natural teeth and implants were tested using commercially available PCR test for detection of 5 periodontal pathogens: Aa, Pg, Pi, Tf and Td. The obtained data were statistically analyzed using SPSS for Windows 16.0. **Results:** No statistically significant differences in periodontal parameters were found between natural teeth and implants, except for recession which was smaller in implants. Microbiological findings revealed an average of 3.56 pathogenic bacterial species around teeth and 3.5 bacterial species around implants per patient. Aa was present in 68.8% of the subjects around teeth and in 75% of the subjects around implants. Pg was present in 82% of the subjects around teeth and in 87% of the subjects around implants. For Pi these values were 32% and 25%, for Tf 88% and 94%, for Td 87% and 68%. Pi was detected in 32% of the subjects around teeth and in 25% of the subjects around implants. These differences between the groups were not statistically significant. Strong correlation between the time of implant placement and the presence of Td was found, a moderate correlation was found for Aa and only weak correlation for Pi and Tf. Strong correlation was observed between the presence of the same bacterial species around teeth and implants for all tested periodontal pathogens ($p<0.05$). X-ray and PD revealed bone resorption around 4 from 49 implants, amounting to 8% of periimplantitis cases. All 3 patients had very high plaque and inflammation values. Of these, 2 harbored very high, and one harbored low levels of all tested periodontal pathogens. **Conclusion:** The combination of high plaque and bleeding values together with the presence of periodontal pathogens seems to be conductive to periimplantitis.

Received: March 28, 2012
Accepted: May 16, 2012

Address for correspondence
Assistant Prof. Andrej Aurer DMD, PhD
University of Zagreb
School of Dental Medicine
Department of Periodontology
Gunduliceva 5
Tel: + 385 1 4802 155
Fax: + 385 1 4802 159
aurer@sfzg.hr

Key words
Peri-Implantitis; Dental Implants; Dental Plaque; PCR

References

1 Berglundh T, Lindhe J. Dimension of the periimplant mucosa. Biological width revisited. J Clin Periodontol. 1996 Oct;23(10):971-3.
2 Albrektsson T, Isidor F. Consensus report: Implant therapy. Proc of the 1st European Workshop on Periodontology. Berlin: Quintessence; 1994. p. 365-9.
3 Berglundh, T. Soft tissue interface and response to microbial challenge. In: Lang NP, Lindhe J, Karring T, editors. Implant dentistry. Proceedings from 3rd European Workshop on Periodontology. Berlin: Quintessence; 1999. pp. 153–74.
4 Vervaeke S, De Bruyn H. Peri-implantitis. Rev Belge Med Dent (1984). 2008;63(4):161-70.
5 Gotfredsen K, Berglundh T, Lindhe J. Bone reactions at implants subjected to experimental peri-implantitis and static load. A study in the dog. J Clin Periodontol. 2002 Feb;29(2):144-51.
6 Grossi SG, Genco RJ, Machtei EE, Ho AW, Koch G, Dunford R et al. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. J Periodontol. 1995 Jan;66(1):23-9.
7 D'Ercole S, Catamo G, Tripodi D, Piccolomini R. Comparison of culture methods and multiplex PCR for the detection of periodontopathogenic bacteria in biofilm associated with severe forms of periodontitis. New Microbiol. 2008 Jul;31(3):383-91.
8 Demmer RT, Papapanou PN, Jacobs DR Jr, Desvarieux M. Bleeding on probing differentially relates to bacterial profiles: the oral infections and vascular disease epidemiology study. J Clin Periodontol. 2008 Jun;35(6):479-86.
9 Zhong LJ, Zhang YM, Liu H, Liang P, Murat AR, Askar S. Detection of periodontal pathogens in coronary atherosclerotic plaques. Zhonghua Kou Qiang Yi Xue Za Zhi. 2008 Jan;43(1):4-7.
10 Eklund SA, Burt BA. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. J Public Health Dent. 1994 Winter;54(1):5-14.
11 Guldag MU, Buyukkaplan US, Ay ZY, Katirci G. A multidisciplinary approach to dental erosion: a case report. Eur J Dent. 2008 Apr;2(2):110-4.
12 Akhter R, Hassan NM, Aida J, Zaman KU, Morita M. Risk indicators for tooth loss due to caries and periodontal disease in recipients of free dental treatment in an adult population in Bangladesh. Oral Health Prev Dent. 2008;6(3):199-207.
13 Rams TE, Link CC Jr. Microbiology of failing dental implants in humans: electron microscopic observations. J Oral Implantol. 1983;11(1):93-100.
14 Mombelli A, Feloutzis A, Brägger U, Lang NP. Treatment of periimplantitis by local delivery of tetracycline. Clinical, microbiological and radiological results. Clin Oral Implants Res. 2001 Aug;12(4):287-94.
15 Okuda K. Bacteriological diagnosis of periodontal disease. Bull Tokyo Dent Coll. 1994 Aug;35(3):107-19.
16 Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: a comparison of sites in edentulous and partially edentulous patients. J Periodontal Res. 1989 Mar;24(2):96-105.
17 Martin JA, Page RC, Kaye EK, Hamed MT, Loeb CF. Periodontitis severity plus risk as a tooth loss predictor. J Periodontol. 2009 Feb;80(2):202-9.
18 Chee WW, Mordohai N. Tooth-to-implant connection: a systematic review of the literature and a case report utilizing a new connection design. Clin Implant Dent Relat Res. 2010 Jun 1;12(2):122-33.
19 García L, Tercero JC, Legido B, Ramos JA, Alemany J, Sanz M. Rapid detection of Actinobacillus actinomycetemcomitans, Prevotella intermedia and Porphyromona gingivalis by multiplex PCR. J Periodontal Res. 1998 Jan;33(1):59-64.
20 Takanashi K, Kishi M, Okuda K, Ishihara K. Colonization by Porphyromonas gingivalis and Prevotella intermedia from teeth to osseointegrated implant regions. Bull Tokyo Dent Coll. 2004 May;45(2):77-85.
21 Sumida S, Ishihara K, Kishi M, Okuda K. Transmission of periodontal disease-associated bacteria from teeth to osseointegrated implant regions. Int J Oral Maxillofac Implants. 2002 Sep-Oct;17(5):696-702.
22 Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. Periodontol 2000. 2001;25:21-36.
23 O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol. 1972 Jan;43(1):38.
24 Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975 Dec;25(4):229-35.
25 Joss A, Adler R, Lang NP. Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. J Clin Periodontol. 1994 Jul;21(6):402-8.
26 Serino G, Ström C. Peri-implantitis in partially edentulous patients: association with inadequate plaque control. Clin Oral Implants Res. 2009 Feb;20(2):169-74.
27 Abreu MH, Bianchini MA, Magini RS, Rösing CK. Clinical and radiographic evaluation of periodontal and peri-implant conditions in patients with implant-supported prosthesis. Acta Odontol Latinoam. 2007;20(2):87-95.
28 Renvert S, Roos-Jansåker AM, Lindahl C, Renvert H, Rutger Persson G. Infection at titanium implants with or without a clinical diagnosis of inflammation. Clin Oral Implants Res. 2007 Aug;18(4):509-16.
29 Cheung LK, Leung AC. Dental implants in reconstructed jaws: implant longevity and peri-implant tissue outcomes. J Oral Maxillofac Surg. 2003 Nov;61(11):1263-74.
30 Lang NP, Berglundh T, Working Group 4 of Seventh European Workshop on Periodontology. Periimplant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011 Mar;38 Suppl 11:178-81.