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# Nalaz subgingivne mikroflore kod pacijenata s implantatnoprotetičkim radovima: Preliminarno izvješće

## *Subgingival Microflora in Patients with Implant-Supported Restorations: Preliminary Report*

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### Sažetak

Bakterijska flora usne šupljine vrlo je kompleksna. Svraha studije bila je analiza kliničkih parodontnih čimbenika i sastava subgingivne mikroflore kod pacijenata sani-ranih implantatno-protetičkim radovima. Studija obuhvaća 28 pacijenata prosječne dobi od 46,7 godina. Nisu bolovali od parodontnih bolesti, niti su bili na parodontološkoj kontroli nakon insercije implantata. Izmjerena je im srednja vrijednost plak indeksa od  $1,04 \pm 0,84$ , dubina džepova  $2,59 \pm 1,13$  i indeks krvarenja gingive  $1,50 \pm 0,84$ . Uzorkovanjem subgingivne mikroflore papirnatim štapićem izolirano je kod 57% pacijenata pet patogenih anaerobnih bakterija: *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella oralis* i *Campylobacter rectus*. Dobiveni rezultati pokazuju da barem kod 57% pacijenata s do-kazanim potencijalno patogenim bakterijama može nastati periimplantitis i smanjiti uspješnost i biološku trajnost implantatno-protetičke terapije. Nalazi upozoravaju na nužnost stalne stručne parodontološke kontrole spomenutih ispitanika.

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### Ključne riječi

zubne naslage; zubni implantati;

*Actinobacillus actinomycetemcomitans*;

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### Uvod

Bakterijska flora usne šupljine vrlo je kompleksna. Pretpostavlja se da se u njoj nalazi više od 700 različitih bakterija (1). Jedan mililitar sline može sadržavati do  $10^8$  bakterija, a plak i do 100 puta više. Mikrobi iz plaka najvažniji su čimbenik za nastanak i progresiju parodontnih bolesti. Kumulacija

### Introduction

Oral cavity flora is very rich and complex. It can contain over seven hundred different types of bacteria (1). Therefore, one ml of saliva can contain up to  $10^8$  (100 million) bacteria. Plaque contains up to one hundred times more bacteria than the same quantity of saliva. They are responsible for beginning and progres-

zubnog plaka posljedica je loše higijene (2-4). Postoje dvije glavne skupine bakterija u usnoj šupljini. Prvu čine fakultativne vrste koje mogu živjeti s kisikom ili bez njega, drugu čine anaerobne vrste – one ne mogu živjeti u prisutnosti kisika. Bakterije koje za preživljavanje trebaju 21% kisika nazivaju se aerobi. Oni zajedno s fakultativnim i aerobnim bakterijama žive u gingivnim sulkusima ili parodontnim džepovima (5-7).

Jedna od terapijskih mogućnosti sanacije djelomične ili potpune bezubosti jest implantatno-proteička terapija (8-10). Njezin uspjeh ovisi o biomehaničkim čimbenicima i nepostojanju infekcije (11-15). Upalni procesi oko prirodnih zuba u pojavnosti, obliku i posljedicama, vrlo su slični onima oko implantata. Periimplantitis je upalno stanje vezano za submarginalni biofilm i oštećenje nekog i mineraliziranog tkiva oko usatka. Novije studije upozoravaju na veću povezanost količine bakterijskog plaka i perimplantatnog tkiva, negoli između plaka i tkiva oko prirodnog zuba (10). Nije savsvo jasno koja količina i vrsta patogenih bakterija uzrokuje bolest parodonta (16). Čak se i imunološki problematični pacijenti mogu uspješno rehabilitirati implantatima (17).

Svrha studije bila je analiza kliničkog i mikrobiološkog nalaza kod pacijenata saniranih implantatno-proteičkom terapijom.

## Materijali i metode

U studiji je sudjelovalo 28 pacijenata (17 muškaraca i 11 žena) s usađenih 28 implantata u privatnoj stomatološkoj ordinaciji. Od tog broja 16 implantata bilo je ITI (Straumann, Waldenburg, Switzerland) a 12 Implata (Schütz-Dental, Rosbach, Germany). Pacijenti su bili u dobi od 22 do 81 godine (srednja vrijednost 46,7 godina). Implantati su im bili usađeni između 6 i 48 mjeseci prije pregleda (srednja vrijednost  $23,6 \pm 13,1$ ) i opskrbljeni metalokeramičkim krunicama. Obje su čeljusti bile podjednako zastupljene. Devet implantata bilo je usađeno u području prednjih zuba (sjekutići i očnjaci), a 19 u stražnjem dijelu (pretkutnjaci i kutnjaci). Pacijenti su bili zdravi i nisu imali parodontne bolesti. Tijekom posljednja tri mjeseca nisu uzimali antibiotike. Ispitivali su se sljedeći čimbenici: plak indeks (PI) prema Silnessu i Löeu - subgingivni uzorci plaka uzeti su sterilnim papirnatim štapićima; dubina džepova (PD) mjerila se plastičnim kalibriranim parodontnim sondama (Hu Friedy, II, USA) na mezijalnim

sion of periodontal disease. Inadequate oral hygiene increases the accumulation of dental plaque (2-4). There are two major groups of bacteria present in oral cavity. There are species that can live in the presence of oxygen and anaerobes that can not live in the presence of oxygen. Rare species have to have 21% of oxygen for living – these are called aerobic bacteria. Anaerobes live together with facultative aerobes primarily in gingival pockets surrounding teeth (5-7).

One of therapeutic possibilities in treating teeth loss is the implant-supported restoration (8-10). The success of this therapy depends on the biomechanical components as well as on the absence of a possible periodontal infection (11-15). The inflammation process around teeth in its occurrence, form and consequence is similar to the one around implants. Periimplantitis is inflammation linkable with submargin smear layer and with damage of soft and mineralized tissue around the implant. Recent studies are pointing at the more significant correlation between the quantity of bacterial plaque and the state of periimplant tissues than between plaque and tissues around the natural teeth (10). It is not clear what composition and amount of bacteria leads to disease initiation (16). Even the patients with immune problems can be successfully treated with implant-supported restorations (17).

The purpose of this study was to analyze clinical and microbial findings in randomly chosen patients with implant- prosthetic restorations.

## Material and Methods

Twenty eight partially edentulous patients (17 males and 11 females), from a private surgery, aged 22 to 81 (mean age 46.7) participated in this study. Twenty eight implants, 16 ITI (Straumann, Waldenburg, Switzerland) and 12 Implata (Schütz-Dental, Rosbach, Germany) were sampled. The implants were inserted 6 to 48 months before the examination. All of them were restored with metalceramic crowns. Both jaws were equally represented. Out of 28 implants, nine implants replaced maxillary anterior teeth (incisors and canines) and nineteen posterior teeth (molars and premolars). The patients were in good general health, without recorded periodontal disease. The subjects did not take antibiotics during the last three months. The following parameters were recorded: plaque index (PI) – according to Silness and Löe, subgingival plaque samples were obtained using a sterile curette; probing pocket depths (PD) - measurements were taken with a calibrated standard probe (Hu Friedy, II, USA) on mesial and distal sub-

i distalnim stranama impalntata, a indeks krvarenja (GBI) određen je prema Mühlemannu i Sonu.

Uzorci za mikrobiološku analizu uzeti su papirnatim štapićima (Absorbent paper points, ISO COLOR, REF A022R) metodom ubriska i to tako da je štapić držan 10 sekundi u subgingivnom području usatka i zatim uronjen u anaerobni transportni medij (WMGA) do prijenosa u mikrobiološki laboratorij unutar dva sata. Uzorci su nasađeni na neselektivni i selektivno obogaćeni medij. Neselektivni medij koristio se za izolaciju fakultativnih anaerobnih bakterija i gljivica (krvni agar, Becton Dickinson, Sparks, USA) i striktno anaerobnih bakterija (Columbia agar, Becton Dickinson, Sparks, USA). Striktno anaerobne patogene bakterije i *Actinobacillus actinomycetemcomitans* izolirani su na selektivnom mediju (Zambon, Hunt, Mandel and Korman mediji, Becton-Dickinson, Sparks, USA). Mediji su, ovisno o vrsti, inkubirani na 35°C tijekom 24 do 72 sata, ili 7 dana. Za izolaciju fakultativnih anaerobnih bakterija i *Actinobacillus actinomycetemcomitans* inkubacija je provedena u atmosferi obogaćenoj ugljičnim dioksidom, uporabom natrijeva karbonata, citrične kiseline i vode. Izolacija striktnih anaeroba (parodontnih patogena) obavljena je u anaerobnim posudama. Anaerobni uvjeti postignuti su uporabom vrećica BBL Gaspak (Becton Dickinson, Sparks, USA). Nakon inkubacije svi su mediji vizualno pregledani. Uzastopnim sađenjem na neselektivne medije čiste su kulture stvorile makroskopski različite kolonije, čije su vrste identificirane testovima API 20A ili sustavom RAPID ID 32A (BioMerieux).

## Rezultati

Nije pronađena razlika u analiziranim čimbenicima između ispitanika s obzirom na spol. Srednje vrijednosti plak indeksa (PI), dubine džepova (DP) i indeks krvarenja gingive (GBI) prikazane su u Tablici 1. Većina izoliranih mikroorganizma klasificirana je prema vrsti i potencijalnoj patogenosti (Slika 1.). Od 28 pacijenata, kod 16 (57,1%) su pronađene potencijalno patogene bakterije, a 12 osoba (42,9%) nije imalo ni jednu testiranu patogenu bakteriju u uzorku plaka. Kod osoba s pozitivnim nalazom najčešće su pronađene bakterije *Actinobacillus actinomycetemcomitans* i *Fusobacterium nucleatum* (8 ispitanika). U nalazu triju osoba identificiran je samo *Actinobacillus actinomycetemcomitans*, a kod dviju

gingival sites of each implant; the gingival bleeding index (GBI) - according to Mühlemann and Son.

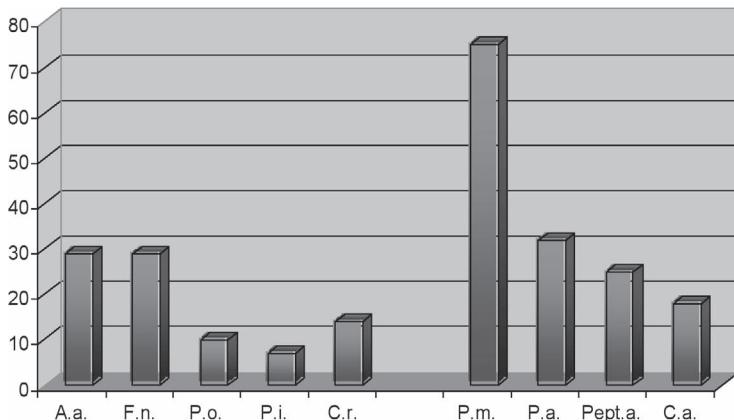
Samples for microbiological analysis were taken with paper-points (Absorbent paper points, ISO COLOR-CODED, REF A 022R) using the smear method. Paper point was held in subgingival region for ten seconds and was then immersed into anaerobic transport medium (WMGA) and delivered at room temperature to the microbiological laboratory within the two-hour period. The samples were planted onto nonselective and selective enriched media. The nonselective media were used for isolation of facultative anaerobic bacteria and fungi (blood agar, Becton Dickinson, Sparks, USA) and strictly anaerobic bacteria (Columbia agar, Becton Dickinson, Sparks, USA). Strictly anaerobic pathogenic bacteria and *Actinobacillus actinomycetemcomitans* were isolated on the selective media (media according to Zambon, Hunt, Mandel and Korman). Media were incubated at 35°C during 24-72 hours, or the period of seven days, depending on the type of medium. Media for the isolation of facultative bacteria and *A. actinomycetemcomitans* were incubated in the atmosphere of approximately 10% CO<sub>2</sub>, created using sodium hydrocarbonate, citric acid and water. The isolation of the strictly anaerobic bacteria (putative periodontal pathogens) was achieved in the anaerobic jars. The anaerobic atmosphere was obtained using BBL Gaspak bags (Becton Dickinson, Sparks, USA). After the incubation, all the media were visually inspected. Pure cultures were created of all macroscopically different colonies by repeated plating on the nonselective media and then identified to the species level using API 20A tests or RAPID ID 32A system (BioMerieux) with manual reading, or using mini API SPECIAL 2000 (BioMerieux).

## Results

Mean values of plaque index (PI), probing pocket depth (PD) and gingival bleeding index (GBI) are shown in Table 1. The majority of isolated microorganisms were classified according to the species and potential pathogenic features in the oral cavity (Figure 1). Out of 28 patients, in 16 (57.1%) potentially pathogenic bacteria were isolated, while the other 12 patients (42.9%) had no pathogenic bacteria in the sulcus sample. In patients positive of pathogenic bacteria, the most frequent bacteria found were *A. actinomycetemcomitans* and *Fusobacterium nucleatum* (8 subjects or 50%). In 3 subjects *A. actinomycetemcomitans* was found as the only pathogen, as was *Fusobacterium nucleatum* in 2 patients.

**Tablica 1.** Deskriptivna statistika za PD, GBI i PI.  
**Table 1** Descriptive statistic of PD, GBI and PI.

	Minimum	Maksimum • Maximum	Sr. vrijednost • Mean	Std. Devijacija • Std. Deviation
PD	1,00	5,00	2,59	1,13
GBI	0	3,00	1,50	0,84
PI	0	3,00	1,04	0,84



Legenda:

**Patogene fakultativne bakterije:**  
A.a. *Actinobacillus actinomycetemcomitans*  
**Patogene anaerobne bakterije:**  
F.n. *Fusobacterium nucleatum*  
P.o. *Prevotella oralis*  
P.i. *Prevotella intermedia*  
C.r. *Campylobacter rectus*

**Ne-patogene anaerobne bakterije:**  
P.m. *Prevotella melaninogenica*  
P.a. *Porphyromonas asacharolytica*  
Pept.a. *Peptostreptococcus asacharolitica*  
**Gljivice:**  
C.a. *Candida albicans*

Legend:

**Potentially pathogenic anaerobic bacteria:**  
A.a. *Actinobacillus actinomycetemcomitans*  
F.n. *Fusobacterium nucleatum*  
P.o. *Prevotella oralis*  
P.i. *Prevotella intermedia*  
C.r. *Campylobacter rectus*

**Non-pathogenic anaerobic bacteria:**  
P.m. *Prevotella melaninogenica*  
P.a. *Porphyromonas asacharolytica*  
Pept.a. *Peptostreptococcus asacharolitica*  
**Fungus:**  
C.a. *Candida albicans*

**Slika1.** Frekvencija (%) bakterijskih izolata u subgingivnim uzorcima pacijenata.  
**Figure 1.** Distribution of bacteria findings.

samo *Fusobacterium nucleatum*. *Prevotella intermedia* nađena je kod dvoje ispitanika, a *Campylobacter rectus* kod četvero. Ostali pacijenti imali su više od jedne spomenute bakterije. Kod devet osoba istodobno su izolirane dvije bakterije. Ni jedan ispitanik nije imao u nalazu tri ili više potencijalno patogenih bakterija. Nije nađena povezanost tipa bakterije i vremena usadnje, odnosno vrste implantata. *Prevotella melaninogenica* kao nepatogeni anaerob nađena je u 75% uzetih uzoraka. *Candida albicans* izolirana je u pet uzoraka (18%). Grupa Viridans, *Streptococci* i saprofitna *Neisseria* dominirali su u odnosu prema fakultativnoj aerobnoj mikroflori.

*Prevotella intermedia* was found in 2 and *Campylobacter rectus* in 4 patients. The other patients had more than one of the previously mentioned bacteria. In 9 patients two bacteria were simultaneously isolated. None of the samples contained three or more bacterial species. *Prevotella melaninogenica* was isolated in 75% samples. A fungus (*Candida albicans*) was isolated in five samples (18%). From the facultative aerobic microflora, Viridans group streptococci and *Saprophytic neisseria* were predominant. The type of bacteria does not depend on the time of implant insertion. There was no difference in parameters observed between male and female.

## Rasprava

Mnogo je sličnosti u mikroflori parodonta i peri-implantatnog tkiva. Oba imaju potencijal koji može uzrokovati progresivnu destrukciju potpornih struktura oko zuba ili implantata (18,19). Zna se da je kod osoba djelomične ozubljenosti kumulacija plaka na zubima slična onoj oko implantata. Parodontni džepovi djeluju kao spremnik za kolonizaciju bakterija na površini usatka (20). Koka i suradnici (21) ustanovili su da se kod pacijenata s djelomičnom bezubostim kolonizacija površine implantata baterijama s okolnih zuba događa do 14 dana od kirurškog otvaranja implantata. U studiji Kalykakisa i suradnika broj bakterija je veći oko implantata inseriranih prije 3 do 4 godine, negoli prije 1 do 2 godine (19). Njihov nalaz kliničkih parodontnih parametara bolji je od naših rezultata u kojima nije dokazana povezanost količine potencijalno patogenih organizama s vremenom usadnje i tipom implantata.

Naime, dentalni implantati svojim materijalom, oblikom i površinom svojevrsni su rizik za taloženje biofilma. Sissons i suradnici (22) i Ichikawa sa suradnicima (23) tvrde da hrapava hidroksilapatitna površina (HA) implantata pojačava taloženje bakterija. No, Griffin i Cheung imaju stopostotni uspjeh kod pacijenata s implantatima presvučenima hidroksilapatitom (24). Davis i suradnici (25) izmjerili su oko pojedinačnih krunica na implantatima s HA površinom srednju vrijednost dubine džepova (PD) od 2 mm. S druge strane titan je stabilniji od HA-a u bilo kojem okolišu. Mombelli i suradnici (26) nisu našli razlike u kliničkim parametrima na različitim tipovima implantata. Očito je pravilna higijena važnija od oblika i površine usatka. Sam stupanj oralne higijene je rizik, rizični čimbenik i/ili pokazatelj mogućeg nastanka parodontne bolesti. Zato su prevencija i uklanjanje plaka temeljni uvjet za oralno zdravlje. Kontrola plaka sama po sebi ograničeno utječe na kliničke parametre vezane za parodontitis, jer ne mijenja mikrobnii sastav u subgingivnom području. Veća količina plaka povećava količinu bakterija *Actinobacillus* i *Fusobacterium* te simptome gingivitisa (27). Heckmann i suradnici (17) dokazali su u najvećoj količini bakterije *Prevotella intermedia* i *Fusobacterium* u kulturi patogenih anaeroba. Lewis i suradnici (28) opisuju bakterije *Prevotella* i *Porphyromonas* kao dva vjerojatno patogena roda u polimikrobnoj oralnoj infekciji. Dubina džepova korelira s nalazom anaerobnih bakterija i s *Porphyromonas gingivalis* (13). Rod bakterija *Fusobacterium* na drugom je mjestu po zastupljenosti u oralnoj mikroflori čovjeka (29). Veže ga se i uz refraktorni

## Discussion

There are many similarities in periimplant and periodontal microflora, both potentially leading to progressive deterioration of the supporting structures (18,19). It is known that accumulation of dental plaque on the implant has about the same rate as the accumulation on the natural teeth. Periodontal pockets serve as reservoirs for the colonization of implant surfaces (20). The findings of Koka et al. (21) suggest that bacteria do colonize implant sites in partially edentulous patients within 14 days after stage II surgery. Kalykakis et al. estimated higher amount of microorganisms around implants inserted 3-4 years ago than around implants inserted 1-2 years ago (19). Values of clinical parameters in their study are lower than in our study. The amount of pathogenic organisms in our subjects did not depend on the type and moment of implant insertion.

Dental implants according to their material, design and surface present a risk for the retentive accumulation of smear layer. Sissons at al.(22) and Ichikawa et al. (23) confirmed that the rougher the hydroxyapatite (HA) surface the more susceptible it is to microbial accumulation. Griffin and Cheung have 100% success in therapy with implants coated with the hydroxyapatite (24). Davis et al. (25) measured mean probing depth of 2.0 mm around the single-tooth implants coated with the hydroxyapatite. On the other hand, titanium is more stable than HA in any environment. Mombelli et al.(26) found no differences in clinical parameters or in microflora composition between two different implant systems. Oral hygiene is obviously more important than shape or surface of the implants. The oral hygiene degree has been the risk, and/or prediction of possible periodontal disease. That confirms the importance of prevention and plaque removing for oral health. Plaque control for itself has limited influence on the clinical parameters connected with periodontitis, because it does not change microbial composition in subgingival area. The general plaque accumulation increases amounts of *Actinomyces* and *Fusobacterium* species with accompanying gingivitis (27). the greatest amount of *Prevotella intermedia* and *Fusobacterium* in the culture of pathogenic anaerobe Among cultivable pathogenic anaerobic species, Heckman et al. (17) have proved more frequent isolation and greater proportions of *P. intermedia*, and *Fusobacterium* species. Lewis et al. (28) are not sure that two species, *Prevotella* and *Porphyromonas*, are pathogen in polymicrobial oral infection. The pocket depth correlates with anaerobic bacteria and with *Porphyromonas gingivalis* (13). The

parodontitis (30). No, Mombelli i suradnici (26) nisu našli *Fusobacterium* u prvih šest mjeseci nakon ugradnje implantata bezubim pacijentima. Antibiotička terapija i čišćenje parodonta smanjuje broj koloniziranih mesta (31). *Actinobacillus actinomycetemcomitans* najčešće je sastavni dio sporadičnog nalaza. U našoj studiji *Actinobacillus actinomycetemcomitans* i *Fusobacterium nucleatum* bile su najčešće pronađene bakterije. Aerobna mikroflora naših ispitanika slična je onoj u ranijoj studiji (32). Ericsson i suradnici (33) ocjenjivali su parodontne čimbenike kod pacijenata s mostovima sidrenima na implantatu i prirodnom zubu. Izmjerili su srednju vrijednost dubine džepova od 3,3 mm oko usatka. Rutar i suradnici (13) i Haffajee (31) našli su oko implantata dubinu džepova veću od 4 mm. Srednje vrijednosti PI (1,04), GBI (1,5) i PD (2,6) indeksa kod naših ispitanika govore o stanju njihova parodonta u trenutku istraživanja. Kako ni jednoga ispitanika nije liječio ili nadzirao parodontolog prije implantatno-protezičke terapije i nakon nje, ne može se govoriti o njezinu utjecaju na stanje parodonta. Tanner i suradnici (16) klinički su pratili 13 zdravih osoba tijekom 6 do 12 mjeseci i uočili male promjene srednje vrijednosti dubine džepova. Temeljem mikrobioloških nalaza (*Actinobacillus actinomycetemcomitans* kod četiri osobe) zaključili su da predominantna mikroflora sadržava neke bakterije vezane uz gingivitis, ali i neke odgovorne za progresivni parodontitis na prirodnim zubima. U drugoj studiji istih autora, kolonizacija *Campylobacter rectus* čimbenik je mogućeg gubitka pričvrstka (27). *C. rectus* dominantana je vrsta u području na kojem je obolio parodont. Bolje rezultate dobili su Ali i suradnici (34). Mjerili su vrijednosti parodontnih čimbenika godinu dana nakon ugradnje implantata (GBI = 0.3-0.4, PD = 0.2 - 0.9 mm). Da nalaz bakterija oko implantata ne znači odmah i patološki proces, dokazali su Mengel i suradnici (35). *Actinobacillus actinomycetemcomitans* i *Porphyromonas gingivalis* nisu dokazani ni nakon jedne godine od ugradnje implantata kod pacijenta s kroničnom parodontnom bolesti. Rezultati Leonhardta i suradnika (36) također potvrđuju činjenicu da spomenute bakterije oko implantata ne moraju uvijek korelirati s lošom higijenom. Dapače, oni tvrde da su *A. actinomycetemcomitans* i *Porphyromonas gingivalis* normalna mikroflora usne šupljine. Prevalencija ispitivanih parodontnih patogena kod odraslih zdravih i oboljelih Rumunja i Kamerunaca slična je onoj u zapadnoj Europi i SAD-u (34, 37). Ali, neobjektivno je usporedjivati nalaze rađene različitim tehnikama izolacije bakterija (34, 38).

*Fusobacterium* species is the second most frequently found anaerobic microbial group in human oral microflora (29). It is implicated in refractory periodontitis (30). However, Mombelli et al. (26) did not find the *Fusobacterium* in the bacterial microflora during the six months of observation period of newly inserted implants in toothless patients. Antibiotics and professional plaque control reduced the number of colonized places (31). In general, *A. actinomycetemcomitans* has been sporadically detected. In our findings *A. actinomycetemcomitans* and *F. nucleatum* were most frequently found. Aerobic microflora was similar to our previous study (32). Ericsson et al. (33) examined periodontal parameters in subjects with bridges supported by the teeth and implants. They found the mean probing depth of 3.3 mm around the implants. Rutar et al. (13) and Haffajee (31) measured more than 4.0 mm probing depth around the implants. The mean values of PI (1.04), GBI (1.5) and of PD (2.6) in our patients suggest their periodontal conditions at the moment of examination. Since they were not regularly periodontally controlled before and after implant-prosthetic therapy, it is not possible to talk about the influence of restoration on the state of periodont. Tanner et al. (16) clinically monitored thirteen healthy subjects during 6-12 month and detected small changes in mean pocket depths. Based on their microbiological findings (*A. actinomycetemcomitans* in four subjects), they concluded that the predominant microflora of putative active subjects included some species previously associated with gingivitis, and some species previously associated with progressive periodontitis in natural teeth. In another study of the same authors, their results suggest, amongst others, colonization of *C. rectus*, as condition able to initiate periodontal loss (27). *C. rectus* was the major species characterizing sites converting from periodontal health to disease. Better results were obtained by Ali et al. (34). They measured the values one year after insertion of the implants (GBI = 0.3-0.4, PD = 0.2 - 0.9 mm). The fact that the microbiological findings around the implants need not necessarily be pathological was proved by Mengel et al. (35). After one year of implants insertion neither *A. actinomycetemcomitans* nor *Porphyromonas gingivalis* were detected in adult patients with chronic periodontitis. The results of Leonardt et al. (36) confirmed that the mentioned bacteria should not correlate with the pure oral hygiene around the implants. On the contrary, they affirmed *A. actinomycetemcomitans* and *Porphyromonas gingivalis* as normal oral findings. The prevalence of the investigated periodontal pathogens in healthy

Ako je svrha održati stabilno i trajno oralno zdravlje, a time i funkciju trajnost protetičke terapije, potrebno je kombinirati dobru osobnu higijenu i profesionalnu instrumentaciju. Prema mišljenju Hellströma i suradnika (39) stručno i često čišćenje zuba i savjesna samokontrola plaka znatno utječe na patogenu subgingivnu mikrofloru.

## Zaključak

Funkcijska trajnost implantatnoprotetičkog rada ovisi o biostatici rada i stanju periimplantatnog područja. Plak i mikrobnna infekcija mogu uzrokovati upalu u subgingivnom području, kako prirodnog zuba tako i implantatnoprotetičkog rada. Zato je uvjet za oralno zdravlje i terapijsku trajnost protetičkih radova ponajprije prevencija parodontnih bolesti i uklanjanje plaka te pravodobno otkrivanje i uklanjanje potencijalno patogenih bakterija. Funkcijska trajnost analiziranih radova razmjerno je kratka da bi se govorilo o uspješnosti terapije, to jest o prognozi. Sastav mikrobnog nalaza i povećani parodontni parametri u ovoj studiji svakako zahtijevaju parodontnu terapiju i daljnju mikrobiološku kontrolu ispitanika. Odgovornost protetičara za trajnost provedene terapije nezamisliva je bez suradnje s parodontologom, budući da samokontrola plaka ima ograničeni učinak. Ova pokusna studija i preliminarni rezultati upozoravaju da je potrebno stalno timski pratiti implantatnoprotetičke pacijente.

and diseased adult Romanian as well as Cameroonian adults was similar to those reported for adults in the western world (34, 37). However, the comparison between findings depends on whether they were obtained by checkerboard hybridization or by traditional culture technique (34, 38).

If it is a goal to maintain stable and permanent oral health and the optimal clinical durability of implant–prosthetic restorations, combination of personal and professional cleaning should be done. According to Hellström et al.(39), professionally performed and frequently repeated tooth cleaning with careful self-performed plaque control has a positive influence on the reduction and effect of pathogenic subgingival microflora.

## Conclusion

Functional durability of implant-prosthetic constructions depends on oral biomechanics and periodontal indexes. Plaque and microbial infection lead to inflammation both in subgingival areas around natural tooth and around implant-prosthetic appliances. That speaks for the importance of prevention of periodontal disease, plaque cleaning and early detection and elimination of potentially pathogenic bacteria. Functional durability of analyzed constructions is relatively short to speak about the successful therapy or about the further prognosis. Composition of microbiological findings and increased values of periodontal parameters in our study suggest periodontal therapy regularly and further microbiological monitoring of patients. The responsibility of prosthodontist is untenable without cooperation with periodontist, because plaque self-control has minor effect. This pilot study and preliminary results point to the need of longitudinally team monitoring of implant-prosthetic patients. Isolated species should not, but could cause perimplantitis.

## Abstract

The composition of oral cavity flora is very complex. The purpose of this study was to analyze the clinical periodontal parameters and the composition of the subgingival microflora in patients with implant-supported restorations. 28 randomly selected patients were included in the study, mean age 46.7; they had no medical record of periodontal diseases and were not periodontally controlled since the placement of the implants. Mean plaque index was  $1.04 \pm 0.84$ , probing depth  $2.59 \pm 1.13$  and gingival bleeding index  $1.50 \pm 0.84$ . By method of paper-point sampling of subgingival microflora, *Actinobacillus actinomycetemcomitans*, and four other conditionally pathogenic anaerobic bacteria - *Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella oralis* and *Campylobacter rectus* in 57% patients were found. These results indicate that the findings of plaque and colonization of potentially pathogenic bacteria in 57% of the patients might cause perimplantitis and might lower the success rate of the biological durability of implant-supported restorations, indicating the need for professional control of the subjects.

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## References

- Schwarzkopf A. Hygiene in der Zahnheilkunde. Swiss Dent. 2001;22(1):5-7.
- Aurer-Koželj J. Osnove kliničke parodontologije. Zagreb: Medicinska naklada; 1992.
- Lindhe J, Karring T, Lang NP, editors. Klinička parodontologija i dentalna implantologija. Zagreb: Globus; 2004.
- Bakdash B. Oral hygiene and compliance as risk factors in periodontitis. J Periodontol. 1994;65(5 Suppl):S539-44.
- Murray PR, Beron EJ, editors. Manual of clinical microbiology. Washington: American Association for Microbiology; 1999.
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. Baltimore: William & Wilkins; 1994.
- Wennstrom JL, Dahlen G, Svensson J, Nyman S. Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius: predictors of attachment loss? Oral Microbiol Immunol. 1987;2(4):158-62.
- Müller HP, Eger T, Lobinsky D, Hoffmann S, Zöller L. Risikofaktor Actinobacillus actinomycetemcomitans in unterschiedlichen Populationen. Dtsch Zahnärztl Z 1996; 51(9):528-32.
- Spiekermann H. Implantologie. Stuttgart, New York: Thieme; 1994.
- Živko-Babić J. Fiksna protetička suprastruktura na implantatima. In: Knežević G et al. Osnove dentalne implantologije. Zagreb: Školska knjiga; 2002. p. 54-60.
- Ivaniš T, Živko-Babić J, Komar D, Čatović A. Relationship between bite forces and main anthropometric dimensions. Coll Antropol. 1996;20(2):377-86.
- Zivko-Babic J, Panduric J, Jerolimov V, Mioc M, Pizeta L, Jakovac M. Bite force in subjects with complete dentition. Coll Antropol. 2002;26(1):293-302.
- Rutar A, Lang NP, Buser D, Burgin W, Mombelli A. Retrospective assessment of clinical and microbiological factors affecting periimplant tissue conditions. Clin Oral Implants Res. 2001;12(3):189-95.
- Uribe R, Penarrocha M, Sanchis JM, Garcia O. Marginal peri-implantitis due to occlusal overload. A case report. Med Oral. 2004;9(2):160-2.
- 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;17(5):696-702.
- Tanner A, Kent R, Maiden MF, Taubman MA. Clinical, microbiological and immunological profile of healthy, gingivitis and putative active periodontal subjects. J Periodontal Res. 1996;31(3):195-204.
- Heckmann SM, Heckmann JG, Linke JJ, Hohenberger W, Mombelli A. Implant therapy following liver transplantation: clinical and microbiological results after 10 years. J Periodontol. 2004;75(6):909-13.
- Ellen RP. Microbial colonization of the peri-implant environment and its relevance to long-term success of osseointegrated implants. Int J Prosthodont. 1998;11(5):433-41.
- Kalykakis GK, Mojon P, Nisengard R, Spiekermann H, Zafiroopoulos GG. Clinical and microbial findings on osseointegrated implants; comparisons between partially dentate and edentulous subjects. Eur J Prosthodont Restor Dent. 1998;6(4):155-9.
- Saito A, Hosaka Y, Sekiguchi K, Kigure T, Isobe S, Shibusawa Y, et al. Responses of peri-implant tissues to undisturbed plaque formation in dogs: clinical, radiographic, and microbiological findings. Bull Tokyo Dent Coll. 1997;38(1):13-20.
- Koka S, Razzoog ME, Bloem TJ, Syed S. Microbial colonization of dental implants in partially edentulous subjects. J Prosthet Dent. 1993;70(2):141-4.
- Sissons CH, Wong L, Shu M. Factors affecting the resting pH of in vitro human microcosm dental plaque and *Streptococcus mutans* biofilms. Arch Oral Biol. 1998;43(2):93-102.
- Ichikawa T, Hirota K, Kanitani H, Miyake Y, Matsumoto N. In vitro adherence of *Streptococcus constellatus* to dense hydroxyapatite and titanium. J Oral Rehabil. 1998;25(2):125-7.
- Griffin TJ, Cheung WS. The use of short, wide implants in posterior areas with reduced bone height: a retrospective investigation. J Prosthet Dent. 2004;92(2):139-44.
- Davis DM, Watson RM, Packer ME. Single tooth crowns supported on hydroxyapatite coated endosseous dental implants: a prospective 5-year study on twenty subjects. Int Dent J. 2004;54(4):201-5
- Mombelli A, Buser D, Lang NP. Colonization of osseointegrated titanium implants in edentulous patients. Early results. Oral Microbiol Immunol. 1988;3(3):113-20
- Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent RL. Microbiota of health, gingivitis and initial periodontitis. J Clin Periodontol. 1998;25(2):85-98.
- Lewis MA, Carmichael F, MacFarlane TW, Milligan SG. A randomised trial of co-amoxiclav (Augmentin) versus penicillin V in the treatment of acute dentoalveolar abscess. Brit Dent J. 1993;175(5):169-74.
- Gaetti-Jardim E Jr, Zelante F, Avila-Campos MJ. Oral species of *Fusobacterium* from human and environmental samples. J Dent. 1996;24(5):345-8.
- Barone A, Sbordone L, Ramaglia L, Ciaglia RN. Microbiota associated with refractory periodontitis. Minerva Stomatol. 1999;48(5):191-201.
- Haffajee AD, Dibart S, Kent RL Jr, Socransky SS. Clinical and microbiological changes associated with the use of 4 adjunctive systemically administered agents in the treatment of periodontal infections. J Clin Periodontol. 1995;22(8):618-27.
- Živko Babić J, Stilinović B, Gašparac I, Jakovac M, Pandurić J, Katuranić M. Aerobna mikroflora subgingivnoj području protetskih pacijenata s implantatima. Acta Stomatol Croat. 2002;36(4):433-6.
- Ericsson I, Lekholm U, Bränemark PI, Lindhe J, Glantz PO, Nyman S. A clinical evaluation of fixed-bridge restorations supported by the combination of teeth and osseointegrated titanium implants. J Clin Periodontol. 1986;13(4):307-12.
- Ali RW, Johannessen AC, Dahlen G, Socransky SS, Skaug N. Comparison of the subgingival microbiota of periodontally healthy and diseased adults in northern Cameroon. J Clin Periodontol. 1997;24(11):830-5.
- Mengel R, Stelzel M, Hasse C, Flores-de-Jacoby L. Osseointegrated implants in patients treated for generalized severe adult periodontitis. An interim report. J Periodontol. 1996;67(8):782-7.
- Leonhardt A, Grondahl K, Bergstrom C, Lekholm U. Long-term follow-up of osseointegrated titanium implants using clinical, radiographic and microbiological parameters. Clin Oral Implants Res. 2002;13(2):127-32.
- Ali RW, Velcescu C, Jivanescu MC, Loftus B, Skaug N. Prevalence of 6 putative periodontal pathogens in subgingival plaque samples from Romanian adult periodontitis patients. J Clin Periodontol. 1996;23(2):133-9.
- Papapanou PN, Madianos PN, Dahlen G, Sandros J. "Checkerboard" versus culture: a comparison between two methods for identification of subgingival microbiota. Eur J Oral Sci. 1997;105(5 Pt 1):389-96.
- Hellström MK, Ramberg P, Krok L, Lindhe J. The effect of supragingival plaque control on the subgingival microbiota in human periodontitis. J Clin Periodontol. 1996;23(10):934-40.