Nalaz anaerobnih bakterija oko implantata i homolognog zuba od 2 do 14 godina nakon ugradnje

Anaerobic Bacteria in Implants and Homologous Teeth 2-14 Years after Implantation

Sažetak

Svrha: Svrha studije bila je utvrditi ima li razlike u prisutnosti potencijalno patogenih anaerobnih mikroorganizama oko implantata i homolognog zuba kod pacijenata koji su nakon postavljanja dentalnih implantata bili upućeni u individualni pristup u održavanju oralne higijene. Materijal i postupci: U istraživanju je sudjelovalo 30 ispitanika (10 muškaraca i 20 žena) prosječne dobi 49,6 godina (22–78 godina). Implantati su bili protecitčki opskrbljeni metalkeramičkim krunicama prosječne starosti 5,26 godina (2 – 14 godina). Na kontrolnom pregledu parodontnom sondom zabilježeni su sljedeći indeksi i mjere: aproksimalni indeks plaka (API), indeks krvareće papile (PBI), dubina sondiranja (PD) i recesija gvinge. Rezultati: Rezultati naše studije pokazali su pozitivan API na 30% implantata, a na 70% bio je negativan. Vrijednosti PBI-ja bile su identične vrijednostima API-ja. Izmjerna je prosječna retrakcija mukoze oko implantata od 0,15 mm i prosječna vrijednost dubine sondiranja oko implantata od 2,25 mm. Na homolognim zubima API je bio pozitivan na 78,3% zuba, kao i PBI. Izmjerna je prosječna retrakcija gingive od 1,06 mm i prosječna vrijednost dubine sondiranja od 1,85 mm. U skupini od 30 ispitanika, anaerobne bakterije izolirane su samo na implantatu, kod tri samo na homolognom zubu, a kod dva i na implantatu i na homolognom zubu. Zaključak: Zapaženo je više anaerobnih bakterija na implantatu u odnosu prema homolognom zubu.

Uvod

Prošlo je gotovo pet godina otkako su Albrektsson i sudradnici (1) zaključili da je perimplantitis infekcija sa supuracijom porezana s klinički znatnim i progresivnim krestalnim guštkom kosti nakon faze adaptacije, no čini se da je broj pacijenata s perimplantatnom infekcijom nepromenjeno u porastu (2). Etiologija perimplantitis je kompleksna, a niz rizičnih čimbenika koji utječu na njegov nastanak i progresiju može se objasniti jedino multikausalnim modelom. Ipak, organizacija i rast biofilma na dentalnim implantatima potiče odgovor domaćina koji izaziva razvoj dubine sondiranja oko implantata, a uzbudte i perimplantitisu (3). Biofilm se može definirati kao agregacija jedne ili više različitih skupina mikroorganizama uživenih u matriks koji sami proizvode i pričvršćen je na neku čvrstu površinu (4).
Može se reći da su inicijalne faze razvoja biofilma na zubi i implantatima identične. Pelikula na površini implantata/suprastrukture veoma je slična pelikuli na prirodnim zubima. U prvoj fazi nastanka biofilma Streptococcus mutans čini od 60 do 80 % svih ranih kolonizatora s različitim bakterijskim adhezionima odgovornima za pričlanjivanje na pelikulu. Rast i diverzifikacija biofilma na implantatima donekle se razlikuju od onih na prirodnim zubima, no neki elementi su identični. Na primjer, kolektivna svijest koju bakterije razvijaju posjepšena je stimulirajućim peptidima koji se otpuštaju nakon izloženosti niskom pH (5). Površina implantata poželjno je nepravilna, no upravo to poguđuje rastu biofilma, organizacije koju danas smatramo primitivnim višestaničnim mikroorganizmom (6). Četiri su elementa koja poguđuju rastu i razvoju biofilma na površini dentalnih implantata: (a) slučajni dolazak bakterija nošenih slinom na površinu implantata, (b) inicijalna (reverzibilna) adhezija, (c) kolonizacija površine te (d) snažna adhezija na površinu (7).

Kontrola biofilma jedan je od glavnih preduvjeta za održavanje zdravlja perimplantarnih tkiva, baš kao i za održavanje zdravlja parodonta. Ipak, zbog morfoloških i anatomskih razlika perimplantatnta tkiva podložnja su razvoju inflamacije od parodontalnih, a i oko dentalnih implantata, čini se, updla brže napređuje (8). Niz iz čimbenika, ponajprije na bakterijskoj razini, koji utječu na to u kojoj će meri biofilm biti izazov za domaćinu. Nastanak upravno potiče snažne promjene u sastavu biofilma (9) – uglavnom se povećava ili smanjuje udjel određenih vrsta (10). Te su razlike posebno izražene u slučaju gingivitsa (11), no nepoznate su uloge određenih vrsta unutar biofilma pri nastanku i progressiji perimplantatnog mukozitisa i periimplantitisa. Kako je perimplantitis teška parodontalna bolest koja može završiti progresivnom difuznom destrukcijom potpore kosti i okolnih tkiva, sigurno je vrlo važno na vrijeme identificirati lokalne parametre koji u većoj mjeri utječu na inicijaciju, odnosno na progressiju bolesti (12).

Upravo zbog toga, ovim smo istraživanjem pokušali utvrditi ima li razlike u prisutnosti tzv. potencijalno parodontopathogenih bakterija (Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola) oko implantata i homolognog zuba kod pacijenata koji su nakon postavljanja dentalnih implantata bili upućeni u individualni pristup u održavanju oralne higijene.

The initial phases of biofilm formation on teeth and on implants can be considered identical. The pellicle on the surface of implants/suprastructures is very similar to the pellicle on natural teeth. In the initial phase of biofilm formation, Streptococcus mutans makes up for 60%-80% of all early colonizers, with different bacterial adhesives responsible for the adhesion to the pellicle. Even though the growth and diversification of biofilm are somewhat different on implants than on natural teeth, certain elements remain identical. For example, the collective consciousness that the bacteria develop is enhanced by the mimetizing peptides that are released after the exposure to low pH (5).

The surface of implants is preferably uneven, but that is precisely what favors the formation of biofilm, the organization that is nowadays considered to be a primitive multicellular organism (6). There are four elements that are favorable for the growth and formation of biofilm on the surface of dental implants: (a) random transport of bacteria to the surface of the implant through saliva, (b) initial (reversible) adhesion, (c) colonization of the surface and (d) strong adhesion to the surface (7).

Controlling the biofilm is one of the main prerequisites for keeping the peri-implant tissue, as well as the periodontal tissue, healthy. However, due to morphological and anatomical differences, peri-implant tissue is more prone to developing inflammation than periodontal tissue, and it appears that the inflammation around dental implants progresses faster (8). There is a series of factors, primarily bacteria-related, that affects the extent to which the biofilm will constitute a challenge for the host. The emergence of inflammation leads directly to significant changes in the composition of biofilm (9), primarily in terms of increasing and decreasing the proportion of certain species (10). These differences are especially noticeable when it comes to gingivitis (11), but the roles of certain species within biofilm in developing peri-implant mucositis and peri-implantitis are unknown. Since perimplantitis 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 (12).

Precisely in view of that, in this study we have attempted to establish whether there is a difference in the presence of potentially so-called periodontopathogenic bacteria (Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola) around the implant and homologous tooth in patients who, after having the dental implants placed, received information about an individual approach to maintaining oral hygiene.

Materijali i metode

Ispitanici

U istraživanju je sudjelovalo 30 ispitanika (10 muškaraca i 20 žena) prosječne dobi 49,6 godina (22 – 78 godina). Svi su morali zadovoljiti kriterij da na kontralateralnoj strani od implantata imaju prirodan zub bez protetičkog nadomjesta. Implantati su bili protetički opskrbljeni metalkera-

Materials and methods

Subjects

The study included 30 subjects (10 males and 20 females), whose average age was 49.6 years (ranging from 22 to 78 years). Aside from having implant-prosthetic interventions done, the chosen participants were required to have natural teeth as well. On top of the participant’s implants,
Anaerobic Bacteria in Implants

Dental prostheses in the form of metal-ceramic crowns had been fixed, whose average age before the examination was 5.26 years (ranging from 2 to 14 years). Four implants were placed in the area of front teeth (incisors), and 26 were placed in the back (premolars and molars). The subjects were healthy and did not show clinical signs of periodontal disease. All the participants had signed an informed consent form for participating in a scientific study, approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb.

Methods

The condition of the participant’s tooth-supporting apparatus and of the tissue surrounding the implant was established during an examination. A periodontal probe (Tekno-Medical Optik-Chirurgie, Tuttlingen, Germany) was used to record the following indexes and measurements: the approximal plaque index (API), the papilla bleeding index (PBI), the periodontal pocket probing depth (PD) and the gingival recession.

After disinfecting with the 3% hydrogen peroxide \((\text{H}_2\text{O}_2)\), drying with compressed air and placing dental cotton rolls, paper points (25) (Absorbent Paper Point Pearl Endopia, Pearl Dent, Kyunggi-Do, Korea) vestibularly were used to sample the fluid around the implant and the gingival sulcus fluid around the homologous tooth on the contralateral side. The paper points were kept in the subgingival area of the implant for 90 seconds, after which they were stored in the anaerobic transport medium (Thioglycollate Medium G, Biolab, Budapest, Hungary) until they were transported to the microbiological laboratory, where they were immediately incubated in the same medium at 37°C for three days. The samples were then cultivated on the anaerobic agar base (Columbia Agar Base, Biolife, Milano, Italy) and the bacteria were identified using the protein mass spectrometry method (matrix-assisted laser desorption/ionization time-of-flight mass spectrometer, MALDI-TOF-MS).
Statistical Analysis

The results obtained were analyzed by the statistical program SPSS 21 (IBM, Armonk, USA). The probability of the correlation of anaerobic bacterial findings with the depth of probing was determined by multiple linear regression model (p<0.05), while estimates of statistically significant differences when comparing the homologous tooth and implant were analyzed by t-test on the difference between the two populations. The remaining results describing the characteristics of the sample were processed with the help of descriptive statistics, regarding measures of central tendency, measures of variability and measures of asymmetry (Table 1–7).

| Var/pokazatelji • Var/indices | N  | SD  | AS  | MD  | t     | p  | Donja • Lower 95% | Gornja • Upper 95% |
|-------------------------------|----|-----|-----|-----|-------|----|------------------|------------------|
| Dubina sondiranja (H) • Probing depth (H) | 30 | 2.43 | 7.50 | -1.47 | -2.74 | <0.05 | -2.56 | -0.37 |
| Dubina sondiranja (I) • Probing depth (I) | 30 | 3.29 | 8.97 | | | |

Statistička analiza

Dobiveni rezultati analizirani su statističkim programom SPSS 21 (IBM, Armonk, SAD). Vjerojatnost povezanosti nalaza anaerobnih bakterija s dubinom sondiranja određena je s pomoću modela višestruke linearne regresije (p < 0.05), a procjene statistički značajnih razlika pri usporedbi homoloognog zuba i implantata bile su analizirane t-testom o razlici sredina dviju populacija. Preostali rezultati kojima se opisuju karakteristike uzorka obrađeni su deskriptivnom statistikom, odnosno mjerama centralne tendencije, mjerama varijabilnosti i mjerama asimetrije (tablica 1–7).

| Tablica 1. Apsolutne i relativne frekvencije API-ja i PBI-ja izražene u broju pozitivnih i negativnih nalaza | Table 1 Absolute and relative frequencies of APIs and PBIs expressed in the number of positive and negative findings |
| Apsolutne frekvencije • Absolute frequencies | Postotne frekvencije • Percent frequencies | Kumulativni postotak • Cumulative Percentage |
| PLAK VESTIBULARNO (H) • PLAQUE VESTIBULAR (H) | | |
| Negativan • Negative | 6 | 20,0 | 20,0 |
| Pozitivan • Positive | 24 | 80,0 | 100,0 |
| PLAK ORALNO (H) • PLAQUE ORAL (H) | | |
| Negativan • Negative | 7 | 23,3 | 23,3 |
| Pozitivan • Positive | 23 | 76,7 | 100,0 |
| UPALA VESTIBULARNO (H) • BLEEDING VESTIBULAR (H) | | |
| Negativan • Negative | 6 | 20,0 | 20,0 |
| Pozitivan • Positive | 24 | 80,0 | 100,0 |
| UPALA ORALNO (H) • BLEEDING ORAL (H) | | |
| Negativan • Negative | 7 | 23,3 | 23,3 |
| Pozitivan • Positive | 23 | 76,7 | 100,0 |
| PLAK VESTIBULARNO (I) • PLAQUE VESTIBULAR (I) | | |
| Negativan • Negative | 21 | 70,0 | 70,0 |
| Pozitivan • Positive | 9 | 30,0 | 100,0 |
| PLAK ORALNO (I) • PLAQUE ORAL (I) | | |
| Negativan • Negative | 21 | 70,0 | 70,0 |
| Pozitivan • Positive | 9 | 30,0 | 100,0 |
| UPALA VESTIBULARNO (I) • BLEEDING VESTIBULAR (I) | | |
| Negativan • Negative | 21 | 70,0 | 70,0 |
| Pozitivan • Positive | 9 | 30,0 | 100,0 |
| UPALA ORALNO (I) • BLEEDING ORAL (I) | | |
| Negativan • Negative | 21 | 70,0 | 70,0 |
| Pozitivan • Positive | 9 | 30,0 | 100,0 |

| Tablica 2. T-test o procjeni razlike dubine sondiranja oko implantata i homolognog zuba (mm) | Table 2 T-test for estimating the depth of probe depth around the implant and the homologous tooth (mm). |
| Var/pokazatelji • Var/indices | N  | SD  | AS  | MD  | t     | p  | Donja • Lower 95% | Gornja • Upper 95% |
|-------------------------------|----|-----|-----|-----|-------|----|------------------|------------------|
| Dubina sondiranja (H) • Probing depth (H) | 30 | 2.43 | 7.50 | -1.47 | -2.74 | <0.05 | -2.56 | -0.37 |
| Dubina sondiranja (I) • Probing depth (I) | 30 | 3.29 | 8.97 | | | |

| Tablica 3. T-test o procjeni razlike retrakcije mukoze oko implantata i gingiva oko homolognog zuba (mm) | Table 3 T-test for estimating the difference of mucosa recession around the implant and gingiva around the homologous tooth (mm). |
| Var/pokazatelji • Var/indices | N  | SD  | AS  | MD  | t     | p  | Donja • Lower 95% | Gornja • Upper 95% |
|-------------------------------|----|-----|-----|-----|-------|----|------------------|------------------|
| Retrakcija (H) • Recession (H) | 30 | 2.76 | 2.13 | 1.83 | 4.38 | <0.05 | 0.98 | 2.69 |
| Retrakcija (I) • Recession (I) | 30 | 1.06 | 0.30 | | | | | |
Results

The results of our study have shown a positive API on 30% of the implants and a negative one on 70% of the implants. The PBI values were identical to the API values. The average mucosal retraction measured around the implants was 0.15 mm (ranging from 0 to 5 mm), and the average probing depth was 2.25 mm (ranging from 0 to 6 mm). As regards the homologous teeth, the API and PBI were positive.
Nalaz anaerobnih bakterija oko implantata Savić i sur.

198

1.06 mm (ranging from 0 to 6 mm), and the average probing depth was 1.85 mm (ranging from 1 to 6 mm).

Nadalje, rezultati analize upućuju na statistički značajnu razliku između dubine sondiranja oko implantata i oko homolognog zuba (MD = -1,47; t = -2,74; p < 0,05). U vezi s

Tablica 6. Deskriptivna statistika dubine sondiranja oko implantata prema API-ju

| API (H) | Dubina džepa distalno • Depth probes distal (mm) | Dubina džepa mezijalno • Depth probes mesial (mm) | Dubina džepa vestibularno • Depth probes vestibular (mm) | Dubina džepa oralno • Depth probes oral (mm) |
|---------|--------------------------------------------------|-------------------------------------------------|-------------------------------------------------|------------------------------------------|
| N       | 21                                               | 21                                              | 21                                              | 21                                       |
| A. sredina • A. mean | 2,52                                            | 2,71                                            | 1,57                                            | 2,29                                     |
| Medijan • Median       | 2,00                                            | 3,00                                            | 1,00                                            | 2,00                                     |
| Std. devijacija • Std. Deviation | 1,03                                            | 1,42                                            | 1,03                                            | 0,96                                     |
| Koeficijent asimetrije • Asymmetry coefficient | 0,54                                            | 0,56                                            | 1,92                                            | 0,50                                     |
| Koeficijent zaobljenosti • Curvature coefficient | 0,34                                            | -0,19                                           | 5,55                                            | -0,44                                    |
| Raspon podataka • Data range | 4                                               | 5                                               | 5                                               | 3                                        |
| Minimum               | 1                                               | 1                                               | 0                                               | 1                                        |
| Maksimum • Maximum    | 5                                               | 6                                               | 5                                               | 4                                        |

Positiiv • Positive

| API (H) | Dubina džepa distalno • Depth probes distal (mm) | Dubina džepa mezijalno • Depth probes mesial (mm) | Dubina džepa vestibularno • Depth probes vestibular (mm) | Dubina džepa oralno • Depth probes oral (mm) |
|---------|--------------------------------------------------|-------------------------------------------------|-------------------------------------------------|------------------------------------------|
| N       | 21                                               | 21                                              | 21                                              | 21                                       |
| A. sredina • A. mean | 2,11                                            | 2,11                                            | 2,22                                            | 2,22                                     |
| Medijan • Median       | 2,00                                            | 2,00                                            | 2,00                                            | 2,00                                     |
| Std. devijacija • Std. Deviation | 0,78                                            | 0,78                                            | 1,20                                            | 0,83                                     |
| Koeficijent asimetrije • Asymmetry coefficient | -0,22                                           | -0,22                                           | 1,68                                            | -0,50                                    |
| Koeficijent zaobljenosti • Curvature coefficient | -1,04                                           | -1,04                                           | 3,69                                            | -1,28                                    |
| Raspon podataka • Data range | 2                                               | 2                                               | 4                                               | 2                                        |
| Minimum               | 1                                               | 1                                               | 1                                               | 1                                        |
| Maksimum • Maximum    | 3                                               | 3                                               | 5                                               | 3                                        |

Tablica 7. Deskriptivna statistika retrakcije mukoze oko implantata prema API-ju

| API (H) | Retrakcija vestibularno • Recession vestibular (mm) | Retrakcija oralno • Recession oral (mm) |
|---------|------------------------------------------------------|----------------------------------------|
| N       | 21                                                   | 21                                     |
| A. sredina • A. mean | .24                                               | .00                                    |
| Medijan • Median       | .00                                                  | .00                                    |
| Std. devijacija • Std. Deviation | 1,09                                              | .00                                    |
| Koeficijent asimetrije • Asymmetry coefficient | 4,58                                              |                                      |
| Koeficijent zaobljenosti • Curvature coefficient | 21,00                                             |                                      |
| Raspon podataka • Data range | 5                                               | 0                                      |
| Minimum               | 0                                                   | 0                                      |
| Maksimum • Maximum    | 5                                                   | 0                                      |

Positiiv • Positive

| API (H) | Retrakcija vestibularno • Recession vestibular (mm) | Retrakcija oralno • Recession oral (mm) |
|---------|------------------------------------------------------|----------------------------------------|
| N       | 21                                                   | 21                                     |
| A. sredina • A. mean | .33                                               | .11                                    |
| Medijan • Median       | .00                                                  | .00                                    |
| Std. devijacija • Std. Deviation | 1,00                                              | .33                                    |
| Koeficijent asimetrije • Asymmetry coefficient | 3,00                                              | 3,00                                   |
| Koeficijent zaobljenosti • Curvature coefficient | 9,00                                              | 9,00                                   |
| Raspon podataka • Data range | 3                                               | 1                                      |
| Minimum               | 0                                                   | 0                                      |
| Maksimum • Maximum    | 3                                                   | 1                                      |

retrakcija gingive od 1,06 mm (0 – 6 mm) i prosječna vrijednost dubine sondiranja od 1,85 mm (1 – 6 mm).

Nadalje, rezultati analize upućuju na statistički značajnu razliku između dubine sondiranja oko implantata i oko homolognog zuba (MD = -1,47; t = -2,74; p < 0,05). U vezi s on 78,3% of the teeth. The average gingival retraction measured was 1.06 mm (ranging from 0 to 6 mm), and the average probing depth was 1.85 mm (ranging from 1 to 6 mm).

Furthermore, the results of the analysis indicate the presence of a statistically significant difference between the probe
Anaerobic Bacteria in Implants

Savić et al.

Anaerobic bacteria were found in 12 out of 30 participants (40%), while no potentially pathogenic anaerobic bacteria were found in the remaining 18 participants (60%). Out of 12 participants, in 7 of them the anaerobic bacteria were present only on the implant, in 3 of them only they were present on the homologous tooth, while in 2 participants the anaerobic bacteria were present on both the implant and the homologous tooth. In those subjects, 13 anaerobic bacteria were found, including Streptococcus anginosus on 2 implants, Propionibacterium acnes on 1 implant, Lactobacillus fermentum on 2 implants and 2 homologous teeth, Lactobacillus spp. on 1 implant and 1 homologous tooth, Bifidobacterium dentium on 1 implant, Veillonella parvula and Prevotella denticola. Reul et al. (14) investigated the existence of statistically significant correlation between probing depth and anaerobic bacterium findings. The results of the homologous tooth analysis indicate the absence of statistically significant correlation, with the model comparing 11.40% of the total variance. No depth of probing in the homologous tooth has a statistically significant association with the presence of anaerobic bacteria. The results of the implants were also analyzed, showing a partial statistically significant correlation between the depth of the probe and the findings of anaerobic bacteria. Specifically, 31.1% of the total variance was interpreted in the model, and in one of the four sites for the measurement of the depth of the probes, they have statistically significant correlation with anaerobic bacterial findings (b2 = 0.637, t = 2.82, p <0.05). Additionally, with the increasing depth of probe, the probability of finding anaerobic bacteria increases.

Discussion

The results of the study have shown that despite the expected potentially pathogenic bacteria, the so-called periodontal pathogens (13) (Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola), potentially pathogenic anaerobic bacteria from the red complex (Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia) have not been isolated in any of the 30 subjects, neither on the implant nor on the homologous tooth.

In 40% of the subjects (14), other anaerobic bacteria have been isolated, including the bacteria from the orange complex. Cortelli et al. (14) demonstrated the trend of a more
češćeg pojavljivanja više anaerobnih bakterija na prirodnim zubima negoli na implantatima, a rezultati našeg istraživanja pokazali su da je više anaerobnih bakterija izoliran na implantatima negoli na homolognim zubima.

Prema dostupnoj literaturi, s obzirom na formiranje biofilme, očekuje se izolacija većeg broja anaerobnih bakterija na prirodnim zubima, odnosno da će ispitnicima kojima su izolirane anaerobne bakterije na prirodnom zubu biti izolirane i na implantatu (15, 16). Neki autori smatraju da su bakterije prirodnih zuba primarni izvor patogene te da izravno djeluju na ishod novopostavljenih implantata (17). No, Schierano i suradnici (18) obavili su analizu biofilme, veza znak na parodontalne patogene oko klinički zdravih zuba i oko implantata, te nisu ustanovili značajne razlike u broju i vrsti bakterija s obzirom na dva mjesta uzorkovanja. Botero i suradnici (19) navode da je postojala značajna povezanost između subgingivnih bakterija na implantatima i susjednim zubima. Na temelju rezultata u našem istraživanju, uočena je prisutnost subgingivnih bakterija na implantatu i susjednom zubu kod dvoje ispitanika.

Koyanagi i suradnici (20) ustanovili su da je mikroflora flora raznolikija u slučaju perimplantitisa u odnosu prema parodontitisu, te da su Fusobacterium spp. i Streptococcus spp. dominanti patogeni u oba stanja. No istaknuli su da je samo kod oboljelih od perimplantitisa izolirana Parvimonas micra, što je u skladu s rezultatima našeg istraživanja.

Neki autori (21) smatraju da je parodontalna bolest povezana s perimplantitom te da su Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia i Treponema denticola bile izolirane u zdravom tkivu, ali i kod osoba s perimplantitnim mimozitom i perimplantitom.

Sumida i suradnici (22) ustanovili su da postoji prijenos Porphyromonas gingivalis i Prevotella intermedia iz parodontnih džepova na područje oko implantata. Stingu i suradnici (23) izolirali su Prevotella intermedia i Prevotella nigrescens kod zdravih osoba i onih s parodontonom bolećim, Prevotella intermedia ubraja se u narančasti kompleks bakterija, zajedno s Fusobacterium nucleatum, Fusobacterium periodonticum, Parvimonas micra, Streptococcus constellatus, Eubacterium nodatum i Campylobacter rectus (24, 25). Prevotella intermedia teško se može razlikovati od Prevotella nigrescens uobičajenim laboratorijskim metodama, uključujući i plinski kromatografiju (26, 27), no može se identificirati i razlikovati od Prevotella nigrescens pomoću metode masene spektrometrije (MALDI-TOF-MS metode). Posljednjih je godina Prevotella nigrescens prihvaćena kao mogući parodontni patogen. Smatra se da potiče produkciju mediadora upale i da s pomoću lipopolisaharida može uzrokovati resorpciju alveolarnih kosti (28). U nekim novijim studijama Prevotella nigrescens izolirana je u značajno većem omjeru na mjesta klinički jače izražene upale pri dužim parodontnim džepovima, što je skladno našoj studiji, a to je dokazano kod jednog ispitanika kojemu je dubina sondiranja vestibularno iznosila 5 mm (25). Također je detektirana u većem postotku kod pacijenata s izraženim lokaliziranim i generaliziranim oblikom parodontitisa te generaliziranim agresivnim i krošćnim parodontitisom (29, 30). U skladu s ovom studijom, frequent presence of anaerobic bacteria on natural teeth than on implants, while the results of our study have shown that more anaerobic bacteria have been isolated on implants compared to homologous teeth.

According to the available literature on the subject, in view of the biofilm formation, it is expected that a larger number of anaerobic bacteria will be isolated on natural teeth or that in the subjects in which anaerobic bacteria have been isolated on natural teeth, they will be isolated on the implant as well (15, 16). Some authors believe that bacteria on natural teeth are the primary source of pathogens and that they directly affect the outcome of the newly-placed implants (17). However, Schierano et al. (18) analyzed the biofilm in relation to periodontal pathogens around clinically healthy teeth and around implants and they have not found any substantial differences in terms of the number and type of bacteria considering the two sampling locations. Botero et al. (19) stated that there was a significant connection between the subgingival bacteria in implants and in the neighboring teeth. The results of our study show the presence of subgingival bacteria on the implant and on the neighboring tooth in two subjects.

Koyanagi et al. (20) found that the microbial flora in peri-implantitis is more varied than in periodontitis and that Fusobacterium spp. and Streptococcus spp. are the dominant pathogens in both conditions. However, they found that Parvimonas micra had been isolated only in the patients with peri-implantitis, which is consistent with the results of our study.

Some authors (21) believe that periodontal disease is related to peri-implantitis and that Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia and Treponema denticola were isolated in healthy tissue, but also in individuals with peri-implant mucositis and peri-implantitis.

Sumida et al. (22) found that Porphyromonas gingivalis and Prevotella intermedia are transported from periodontal pockets of healthy teeth onto the area around the implant. Stingu et al. (23) isolated Prevotella intermedia and Prevotella nigrescens both in healthy individuals and in patients with the periodontal disease. Prevotella intermedia species belongs to the orange complex of bacteria, together with Fusobacterium nucleatum, Fusobacterium periodonticum, Parvimonas micra, Streptococcus constellatus, Eubacterium nodatum and Campylobacter rectus (24, 25). It is difficult to identify Prevotella intermedia and distinguish it from Prevotella nigrescens using ordinary laboratory methods, including gas chromatography (26, 27), but that can be achieved using the protein mass spectrometry method (MALDI-TOF-MS method). Prevotella nigrescens has recently been accepted as a possible periodontal pathogen. It is believed that it fosters the production of mediators of inflammation and that its lipopolysaccharide may cause alveolar bone resorption (28). In some other recent studies Prevotella nigrescens was isolated in a substantially larger proportion from the places with a clinically more prominent inflammation associated with deeper periodontal pockets, which was demonstrated in one of the subjects of our study, whose probing depth was 5 mm vestibularly (25). A higher percentage of Prevotella nigrescens was also detected in patients with localized and generalized forms of peri-
rezultati našeg istraživanja također su pokazali da je, uz klinički izraženu upalu oko implantata s vestibularnom dubinom sondiranja od 5 mm, *Prevotella nigrescens* izolirana kod jednog ispitanika.

Lisa Heitz-Mayfield (31) uočila je povezanost između loše oralne higijene, anamnestičkih podataka o parodontitisu i pušenja kao najznačajnijih rizičnih čimbenika za nastanak perimplantitisisa.

Poznato je da hrapavost površine implantata utječe na kolonizaciju biofilma. Titanjska površina s hrapavošću koja je prosječno Ra < 0,088, inhibira kolonizaciju i sazrijevanje biofilma (32). Suprotno tomu, hrapavost površine Ra > 0,2 µm povećava stvaranje biofilma te požaguje nastanak perimplantitisisa (33). Ra < 0,2 µm nema utjecaja na stvaranje supragingivnog i subgingivnog plaka (34) te su zato neki istraživači (35) zaključili da Ra < 0,2 µm nema učinka na mikrofloru.

Istraživanje provedeno 2016. godine dokazalo je da su mikroorganizmi u perimplantantnim lezijama slični onima u parodontnim lezijama, ali da je korelacija između dosadašnjih studija dosta teška zbog dručkih rizičnih čimbenika. Upravo ta spojastom parodontne bolesti razlikuju u raznim dijelovima svijeta na prirodnom zubu biti izolirane i na implantatu (15, 16).

Zaključak

U skupini od ukupno 30 ispitanika nisu izolirane potencijalno patogene anaerobne bakterije iz crvenoga kompleksa (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*). Iz rezultata ovog istraživanja jasno je da su od 30 ispitanika kod njih 12 (40%) izolirane druge vrste anaerobnih bakterija, uključujući i one iz narančastog kompleksa, bilo na zubu, bilo na implantatu. Zapaženo je više anaerobnih bakterija na implantatu negoli na homolognom zubu. Anaerobne bakterije koje su bile istodobno i na implantatu i na homolognom zubu nađene su u manjem broju uzoraka.

S obzirom na formiranje biofilma, moglo se očekivati da će se više anaerobnih bakterija izolirati na prirodnom zubu, ili da će ispitnicima kojima su izolirane anaerobne bakterije na prirodnom zubu biti izolirane i na implantatu (15, 16).

Neki autori smatraju da se mikroorganizmi povezani s nastankom parodontne bolesti razlikuju u raznim dijelovima svijeta i mogu varirati zbog niza čimbenika. Upravo to spoznaja trebala bi potaknuti svaku zemlju da uspostavi vlastiti dentalni mikrobiološki profil kako bi se pripremilo smjernice za provedbu odgovarajućih preventivnih mjera te se u skladu s tim poduzele ciljane terapijske mjere (23).

U svrhu dobivanja što relevantnijih rezultata, potrebna su daljnja istraživanja na što većem broju ispitanika.

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U svrhu dobivanja što relevantnijih rezultata, potrebna su daljnja istraživanja na što većem broju ispitanika.

Sukob interesa

Nije bilo sukoba interesa.

Conflict of interest

None declared
Abstract

Objective: The objective of the study was to establish whether there is a difference in the presence of periodontal anaerobic bacteria around the implants in implant-prosthetic patients who received individual information about maintaining their oral hygiene.

Material and methods: The study included 30 subjects with dental implants and metal-ceramic crowns. A periodontal probe was used to record the approximate plaque index (API), the papilla bleeding index (PBI), the periodontal pocket probing depth (PD) and the gingival recession. The fluid around the implant and the gingival sulcus fluid around the homologous tooth on the opposite lateral side were sampled. Results: The results have shown a positive API and PBI on 30% of the implants and a negative one on 70% of the implants. The average mucosal retraction measured around the implants was 0.15 mm, and the average probing depth was 2.25 mm. The API and PBI were positive on 78.3% of the homologous teeth. The average gingival retraction measured was 1.06 mm, and the average probing depth was 1.85 mm. Anaerobic bacteria were found in 12 out of 30 subjects (40%). Anaerobic bacteria were isolated only on the implant in 7 subjects, only on the homologous tooth in 3 subjects and both on the implant and the homologous tooth in 2 subjects.

Conclusions: Anaerobic bacteria were more abundantly present on implants than on homogenous teeth.

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