

ZNAČAJ PREVALENCIJE PARODONTALNIH PATOGENA U NASTANKU PARODONTITISA

SIGNIFICANCE OF THE PREVALENCE OF PARODONTAL PATHOGENS IN THE OCCURRENCE PARODONTITIS

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Kratak sadržaj

Osnovni princip epidemiologije ukazuje da izražavanje oboljenja predstavlja interakciju domaćina, mikrobnog agensa i faktora okruženja. Izvor za razumevanje patogeneze je identifikovati elemente koji su u ovoj kompleksnoj areni značajni, kao i otkriti redosled događaja koji, nesumljivo, dovode do oboljenja.

Veliki doprinos u istraživanju parodontalnih oboljenja leži u razumevanju i objašnjenju sinteze, metabolizma i mehanizama faktora mikrobine virulencije. Korišćenje specifičnih DNK i RNK sondi nije omogućilo samo oralnu mikrobnu transkripciju, već je doprinelo i brzu analizu kompleksnog sastava mikrobine flore dentalnog plaka-biofilma. Značajnost se ogleda u mogućnosti boljem razumevanju oralne ekologije parodontalnih patogena, kao i u praćenju populacije odabranih patogena. Usavršene tehnike molekularne biologije su doprinele boljem razjašnjenju ćelijskih i molekularnih zbivanja koji su uključeni u zapaljenje i propadanje tkiva parodonta.

Ključne reči: parodontitis, prevalencija parodonlnih patogena, mikrobitna viruljenca

Abstract

The fundamental principle of epidemiology states that a disease is the result of an interaction between the host, microbial agent, and environmental factors. The key to understanding pathogenesis is the identification of the elements of significance in this complex arena and deciphering a sequence of events undoubtedly leading to disease.

A huge contribution in the study of parodontal diseases lies in the comprehension and explanation of the synthesis, metabolism, and pathways of the microbial virulence factors. The use of specific DNA and RNA probes enabled not only oral microbial transcription, but also contributed to a rapid analysis of the complex composition of microbial flora of the dental plaque biofilm. The significance is also reflected in the possibility of better understanding of oral ecology of parodontal pathogens and in the monitoring of the populations of selected pathogens. Advanced techniques of molecular biology contributed to a better elucidation of cellular and molecular events involved in the inflammation and decay of parodontal tissue.

Key words: parodontitis, prevalence of parodontal pathogens, microbial virulence

Uvod

Mnogobrojna istraživanja kod nas i u svetu su pokazala da postoji visoka proporcija parodontitisa i odgojenih parodontalnih patogena.

U USA, jedna studija je otkrila da je 70% parodontitisa inficirano Porhyromonas gingivalis-om, u 50% je dominirala Prevotella intermedia i Eikenella corrodens, 36% sa Campylobacter rectus i 11% sa Actinobacillus actinomycetemcomitans.

U Keniji je prevalenca subjekta i mesta 70% i 50% za Porhyromonas gingivalis, za Prevotella intermedia 100% i 90%, za Actinobacillus actinomycetemcomitans 40% i 28%.²⁰

U Šri Lanki otkriveno je da su Prevotella intermedia, Porphyromonas gingivalis i Actino-

Introduction

Numerous investigations in our country and abroad have demonstrated that there is a high proportion of parodontitis and grown parodontal pathogens.

In the U.S.A., a study has revealed that 70% of the cases of parodontitis were infected with Porphyromonas gingivalis, in 50% the causative agent was Prevotella intermedia, in 36% Campylobacter rectus, and in 11% of the cases Actinobacillus actinomycetemcomitans.

In Kenya, the prevalence of subject and site is 70% and 50% for Porphyromonas gingivalis, 100% and 90% for Prevotella intermedia, and 40% and 28% for Actinobacillus actinomycetemcomitans, respectively.²⁰

bacillus actinomycetemcomitans zastupljeni u 76%, 40% i 15% subjekta. Porphyromonas gingivalis i Prevotella intermedia su bili značajno veći u slučajevima umerenog i uznapredovalog parodontitisa.¹⁹

Istraživanja rađena u Tanzaniji su pokazala da su duboki đžepovi sa visokim nivoom spiroheta, koji napouzdano definišu mesta sa destruktivnim parodontitisom. Prisustvo bakterijskih vrsta u subgingivalnom dentalnom plaku je bilo u vezi sa kliničkim karakteristikama parodonta. Parodontalni patogeni su otkriveni sa rastućom prevalencijom u dubokim parodontalnim đžepovima.²

Istraživači su otkrili u đžepovima >5mm visoke nivoe Porphyromonas gingivalis; Actinobacillus actinomycetemcomitans na 3mm; Prevotella intermedia na 4mm; Eikenella corrodens na 2,7mm i Fusobacterium nucleatum na 2,8mm. Spirohete su otkrivane najčešće, u apikalnoj regiji. Mesta sa supuracijom su pokazivala visok nivo Porphyromonas gingivalis i Prevotelle intermedije. Kod lečenih apsesa nisu nađeni.³

Povećanje subgingivalne temperature rezultira povećanjem Prevotelle intermedia, Actinobacillus actinomycetemcomitans i Porphyromonas gingivalis-a.

Gubitak alveolarne kosti ukazuje na veće prisustvo Prevotelle intermedie.¹

Istraživanja Grossa i sar, 1995 su pokazala da je težak gubitak alveolarne kosti u korelaciji sa prevalencijom bacteroides forsythus i Porphyromonas gingivalis-om. Istovremeno, došli su do rezultata da je gubitak koštane veze udružen sa starenjem, pušenjem i dijabetesom / faktor rizika za gubitak alveolarne kosti je 2,52 i 1,73/.⁶

Više studija se bavilo istraživanjem prisustva bakterija i parodontitisa kod HIV inficiranih pacijenata. Bolest je imala različite nazive. Prvobitno, oblik i crvenilo gingive kod HIV inficiranih pacijenata se odnosio na AIDS-virus gingivitis, a kasnije HIV-gingivitis. Najnoviji naziv za ovu vrstu gingivitisa poznat je kao „linearni gingivalni eritem“. ⁶ Parodontitis otkriven i registrovan kod HIV inficiranih pacijenata rezultirao je sa jako izraženom inflamacijom i bolom gingive. Zbog izraženog progresivnog toka, nazvan je „AIDS virus udružen parodontitis“, a danas kao „HIV parodontitis“. Klinički termin je „nekrotizirajući parodonitis“.⁴

In Sri Lanka, it has been established that Prevotella intermedia, Porphyromonas gingivalis, and Actinobacillus actinomycetemcomitans were present in 76%, 40%, and 15% of subjects, respectively. Porphyromonas gingivalis and Prevotella intermedia were significantly more present in the cases of moderate and advanced parodontitis.¹⁹

Studies conducted in Tanzania have shown that deep pockets contain a high level of spirochetes, unreliably defining the sites with destructive parodontitis. The presence of bacterial species in the subgingival dental plaque was related to the clinical characteristics of the parodontium. Parodontal pathogens were detected with a rising prevalence in deep parodontal pockets.²

Investigators have detected high levels of Porphyromonas gingivalis within the pockets >5 mm; Actinobacillus actinomycetemcomitans at 3 mm; Prevotella intermedia at 4 mm; Eikenella corrodens at 2.7 mm; and Fusobacterium nucleatum at 2.8 mm. Spirochetes were most commonly detected in the apical region. Sites with suppuration demonstrated high levels of Porphyromonas gingivalis and Prevotella intermedia. These were not found in treated abscesses.³

Increased sublingual temperature results in an increased presence of Prevotella intermedia, Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.

Alveolar bone losses indicate a more strongly marked presence of Prevotella intermedia.¹

The studies by Gross et al. in 1995 indicated that severe alveolar bone loss was in correlation with the prevalence of Bacteroides forsythus and Porphyromonas gingivalis. At the same time, they came to the conclusion that bone attachment loss was associated with ageing, smoking, and diabetes (the risk factor for alveolar bone loss was 2.52 and 1.73).⁶

Several studies investigated the presence of bacteria associated with parodontitis in HIV patients. Various terms have been used to denote the disease. The shape and redness of the gums in HIV infected patients were initially described as AIDS-virus gingivitis, and as HIV-gingivitis later. The latest term for this type of gingivitis is „linear gingival erythema“. ⁶ Parodontitis detected and registered in HIV-infected patients resulted in severe inflammation and painful gingiva. Due to its very progressive course it was termed „AIDS virus associated parodontitis“ and today „HIV parodontitis“. The clinical term is „necrotizing parodontitis“.⁴

Studije na nivou mikrobiologije su ukazale na različitu mikrobnu etiologiju parodontitisa. Otkrivena je visoka prevalencija oralnih gljivica.¹⁰ Novija istraživanja su rezultirala podacima da postoji statistička značajnost između kandidijaze i linearнog gingivalnog eritema kod HIV inficiranih homoseksualaca, dok kod HIV inficiranih narkomana nije nađena statistička značajnost. Kod HIV inficiranih osoba može biti povećan nivo Mykoplasma salivarum u oralnoj flori. Parodontitis karakteriše prisustvo tradicionalnih parodontalnih patogena: *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus*, vrste *Eubacterium*, *Porphyromonas gingivalis* i *Prevotella intermedia*. Razvoj bolesti je u direktnoj korelaciji sa stepenom imunosupresije.

Reynolds i sar., su 1989. ispitivali mikrofloru pacijenata obolelih od leukemije. Gubitak epitelnog pripaja je bio u direktnoj vezi sa supragingivalnim stafilokokama i subgingivalnim gljivicama.¹⁴

Rams i sar., su 1991. doveli u vezu značaj prisustva gljivica, enterobakterija i pseudomonasa kod ovih pacijenata. Međutim, pitanje je da li je ovakva flora rezultat primenjene terapije?¹³

Imunosupresija je bila interesantna za istraživanje mikroflore kod pacijenata sa dijabetesom. Istraživanja su bila usmerena na pacijente koji ne kontrolišu ili slabo kontrolišu bolest, i na one koji to rade odgovorno. Cilj je bio utvrditi infekciju parodontalnim patogenima kod jednih i drugih pacijenata. Međutim, veza nije pronađena između kontrolisanog dijabetesa i infekcije parodontalnim patogenima. Kod poboljšanja kontrole dijabetesa i prisustva subgingivalnih mikroorganizama, nadena je povećana proporcija streptokoka u parodontalnim džepovima.¹⁷

Određivanje mnogobrojnih bakterijskih vrsta odgovornih za zdravlje parodonta ili za različit stepen bolesti, baziran je na virulentnosti patogena. Tako je produkcija leukotoksina, kolagenaze, endotoksina, fibroblast inhibitornog faktora, važna za selekciju *Actinobacillus actinomycetemcomitans-a*. Otkriće povećane produkcije kolagenaze, tripsin-like aktivatora, fibrinolizina, proteaza, fosfolipaza, endotoksina, različitih faktora koji omogućavaju leukocitnu funkciju, važno je za *Porphyromonas gingivalis*.¹⁶

Microbiological studies have indicated variable microbial etiology of parodontitis. A high prevalence of oral fungi has been detected.¹⁰ More recent studies have suggested that there is a high degree of statistical significance of the association of candidiasis and linear gingival erythema in HIV infected homosexuals, while there is no statistical significance in HIV infected drug addicts. In HIV infected individuals the level of *Mycoplasma salivarum* may be elevated. Parodontitis is characterized by the presence of traditional parodontal pathogens: *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus*, *Eubacterium species*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. Development of the disease is in direct correlation with the degree of immunosuppression.

Reynolds et al. (1989), investigated oral microflora in leukemia patients. According to their findings, the loss of epithelial attachments was directly related to supragingival staphylococci, and subgingival fungi.¹⁴

Rams et al. (1991), detected a significant association of the disease with the presence of fungi, enterobacteria, and pseudomonas in these patients. However, was the flora such as this the result of the therapy administered?¹³

Immunosuppression was an interesting factor to be assessed in the studies of microflora in diabetic patients. These studies enrolled the patients without control or with poor control of the disease and those who take good care of themselves and their disease. The aim was to identify possible infections with parodontal pathogens in these two patient subpopulations. However, the association between controlled diabetes and infections with parodontal pathogens could not be established. In the patients with improved control of their diabetes and with the presence of subgingival microorganisms, an increased proportion of streptococci in parodontal pockets has been identified.¹⁷

Identification of numerous bacterial species responsible for parodontal health or various disease stages is based on the virulence of pathogens. The production of leukotoxins, collagenase, endotoxins, fibroblast inhibitory factor, is essential for the selection of *Actinobacillus actinomycetemcomitans*. The discovery of increased production of collagenase, trypsin-like activator, fibrinolysin, proteases, phospholipases, endotoxins, various factors enabling leukocytic function, is vital for *Porphyromonas gingivalis*.¹⁶

Ne treba zanemariti i izuzetnu značajnost subgingivalnih mikroorganizama u regulaciji ekspresivnosti bakterijske virulentnosti.

Razumevanje različitih zaštita kod parodontitisa, pripisuju se osnovama imunologije i imunogenetike. U svetu mnogobrojnih virulentnih faktora, odbačena je važnost odbrane u lokalnoj sredini, već se ističu sredina i genetski uslovi u lokalnom oštećenju ili sistemu imunog odgovora. Oni se sagledavaju generalno u procesu razvoja ili progresije parodontitisa. Rapidna progresija i forme parodontitisa koje se teško leče, vezane su za mnogobrojne faktore. Istovremeno je i otkrivanje njihovog patogenog potencijala veoma otežano zbog aktiviranja niza odbrambenih mehanizama.

Inflamatorni odgovor napadnutog tkiva se razlikuje od jednog do drugog subjekta. Ispitanja su pokazala da postoji korelacija između inflamatornog odgovora domaćina i rizičnih pacijenata /rani početni parodontitis, parodontitis dijabetičara idr/. Pušenje, stres, serum-ska antitela ili biohemski markeri inflamacije imaju veliku značajnost u izražavanju bolesti, uz više promenljivih, ali nezavisno od uticaja koji potiču od mikrobne komponente.¹²

Nesumljivo, postoje brojne komponente virulentnosti mikroorganizama, odgovora domaćina i uticaja sredine koji su bitni iz nekog aspekta patogeneze bolesti. Ali, nisu sve komponente važne za početak bolesti. Čvrsti činjenični dokazi ukazuju da su neke komponente od suštinskog značaja za „kritičnu stazu“, na putu ćeljske i molekularne patogeneze u nastanku i manifestaciji bolesti. Uzgred, mnogobrojni biohemski faktori mogu uticati na proces nastanka inflamatornog odgovora parodonta, ali su neki samo opasni tj kritični za proces, dok se samo mali broj može smatrati odgovornim za proces ozdravljenja.⁹

Identifikovani faktori rizika, koji se odnose na fenotip odgovora domaćina, daju mogućnosti za lakše otkrivanje pacijenata visokog rizika. Takođe, razumevanje sredine ili ponašanje može da modifikuje izražavanje oboljenja tj parodontitisa. Mnogobrojne studije su pokazale da klinički zdravi pojedinci imaju parodontitične mikroorganizme koji su non-virulentni klonalni tipovi. Izvesni „virulentni“ klonalni tipovi se povezuju sa nastankom bolesti parodonta. Oni mogu biti otkriveni kod svih članova jedne porodice. Međutim, čak i prisustvo virulentnih

We should not disregard the immense significance of subgingival microorganisms in the regulation of expression of bacterial virulence.

Understanding of various protection pathways in parodontitis can be attributed to the fundamentals of immunology and immunogenetics. In the light of numerous virulent factors, the significance of defense in the local surroundings has been discarded; instead, the environment and genetic conditions are believed to play the principal role in local damage or in the system of immune response. They are both viewed generally during the development or progression of parodontitis. Rapid progression and forms of parodontitis which are difficult to treat are related to numerous factors. At the same time, any determination of their pathologic potential is very complex due to the activation of a sequence of defense mechanisms.

Inflammatory response of an infected tissue differs from one person to another. Various studies have shown that there is a correlation between the host inflammatory response and patient risk factors (early parodontitis, parodontitis in diabetics, and so on). Smoking, stress, serum antibodies, or biochemical markers of inflammation are all very significant in the disease occurrence, with several variables, but independent from the influences exerted by the microbial component.¹²

Undoubtedly, there are numerous components, including microbial virulence, host response, and environmental influences, essential for particular aspects of disease pathogenesis. Firm evidence indicates that some of the components are of vital importance for the „critical pathway“ in the course of cellular and molecular pathogenesis in the occurrence and manifestation of the disease. Moreover, many biochemical factors can modulate inflammatory response of the parodontium, but only a few of them pose a threat to the critical process and a few can be considered responsible for the process of healing.⁹

The identified risk factors related to the host response phenotype enable easier identification of high risk patients. Moreover, the environment or microbial behavior can modify disease manifestations. Numerous studies have demonstrated that clinically healthy individuals can harbor parodontal non-virulent clonal type microorganisms. Certain „virulent“ clonal types are associated with parodontal disease. These can be detected in all the members of a family. However, the presence of virulent clonal types

klonalnih tipova ne rezultira obaveznom pojmom bolesti kod svih inficiranih osoba.

Podaci istraživanja Zambon-a J.J., 1985, ukazuju da je prisustvo visoko leukotoksičnog genotipa *Actinobacillus actinomycetemcomitans* u vezi sa pojavom lokalizovanog parodontitisa kod mlađih osoba.^{21,22}

Iz svega navedenog, dolazi se do zaključka da kliničko izražavanje bolesti zavisi od brojnih faktora, ali da svi faktori nisu jednako značajni. Neki su samo uzročnici npr mikroorganizmi, dok drugi imaju sposobnost promene odgovora domaćina u odbrambenom pravcu ili u pravcu prevelike osetljivosti. Kolonizacija virulentnim klonalnim tipom patogena je zasigurno, neophodnost, ali ne i sigurnost u nastanku oboljenja. Proizilazi da je parodontitis uništenje, razaranje parodonta pod dejstvom specifične bakterijske infekcije kod osetljivog domaćina. Parodontopatična bakterijska flora je neophodna, ali ne i dovoljna za nastanak bolesti.

Epidemiološka istraživanja su pokazala da u reprezentativnim populacijama mnogih modernih društava, nivo kontrole plaka ne doprinosi značajno predviđanju bolesti parodonta.⁸

Neke vrste *Actinobacillus actinomycetemcomitans* ili *Campylobacter rectus* proizvode leukotoksine koji su sposobni da unište neutrofile. Iako je velika uloga virulentnosti mikroorganizama da aktivira odbrambene mehanizme domaćina, neki mikroorganizmi kao npr *Porphyromonas gingivalis* proizvode enzime koji imaju sposobnost degradacije imunoglobulina i komplementa. Može doći do razdvajanja Fc oblasti od bakterijski vezanog IgG, što rezultira sprečevanje fagocitoze i njihovo uništenje. Takođe, mogu da razdvoje C3b komponentu komplementa od ćelijskih zidova, pri čemu se uklanjaju opsoničke sposobnosti komplementa.¹⁵

Gregori i sar. su ukazali na tri vrste mikroorganizama: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* i *Capnocytophaga ochracea* i njihovu povezanost sa nastankom lokalizovanog ranog parodontitisa, uz istovremenu izrazitu degradaciju sve četiri vrste imunoglobulina.⁵

Neke vrste *Actinobacillus actinomycetemcomitans* mogu da daju produkte koji se vezuju za Fc regiju IgC. Tako, ovi molekuli blikiraju fagocitozu pri čemu se javlja normalna povratna inhibicija B ćelija za Ig produkciju.⁷

does not necessarily result in parodontal disease in all infected individuals.

The study data of Zambon J.J. (1985) pointed out the fact that the presence of highly leukotoxic genotype of *Actinobacillus actinomycetemcomitans* could be associated with localized parodontitis in younger individuals.^{21,22}

All of the above could be the basis for the conclusion that a clinical investigation of a disease depends on a multitude of factors of different significance. Some of them can only cause a disease, e.g. microorganisms, while others have the ability to modify host response towards defense or towards increased sensitivity. A colonization with virulent clonal type of pathogens is, naturally, a necessity, but not a certainty in the occurrence of a disease. Parodontitis is the decay, destruction of the parodontium under the impact of a specific bacterial infection in a sensitive host. Parodontopathic flora is necessary but not sufficient for a disease to occur.

In representative populations of many modern societies, epidemiologic studies have shown that the level of plaque control does not contribute in a significant way to the prediction of parodontal disease.⁸

Some species of *Actinobacillus actinomycetemcomitans* or *Campylobacter rectus* produce leukotoxins capable of destroying neutrophils. Although microbial virulence activates host defense mechanisms, some microorganisms (for instance, *Porphyromonas gingivalis*) are able to produce the enzymes which can decompose immunoglobulin and complement. Fc domain can be severed from the bacteria-bound IgG, which results in the prevention of phagocytosis and their destruction. Moreover, the enzymes can detach the C3b component from the cellular walls, removing thus the opsonic abilities of the complement.¹⁵

Gregory et al. have stressed three types of microorganisms: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Capnocytophaga ochracea*, and their association with localized, early parodontitis with simultaneous severe degradation of all four types of immunoglobulin.⁵

Some species of *Actinobacillus actinomycetemcomitans* are able to produce the products which bind to the Fc domain of IgC. These molecules, therefore, block phagocytosis, which is accompanied with normal feedback inhibition of Ig production by B cells.⁷

Mnogi mikroorganizmi koji koloniziraju parodontalni sulkus i đžepove, su razvili visoko specijalizovane enzimske sposobnosti npr plazminogene /preko aktivacije kinina/, imunoglobuline A i G i komplementne proteine domaćina, kao što je C3. Pored sposobnosti da razlažu proteine domaćina, oni ubrzavaju proces inflamacije i tako onemogućavaju odbrambene sposobnosti domaćina. Vremenom, plak postaje sve kompleksniji, a njegovo sazrevanje u subgingivalnoj sredini je neometano.¹⁸

Mnogobrojni mikroorganizmi obezbeđuju proizvode značajne za održavanje drugih u kompleksnoj sinergističkoj sredini sa obiljem hranljivih sastojaka. Mikroorganizmi plaka žive kao deo kompleksnog mikrobnog ekosistema, pri čemu mikrobna raznolikost omogućava njihov rast i razmnožavanje.¹¹

Many microorganisms, which colonize the periodontal sulcus and pockets, have developed highly specialized enzymatic abilities, e.g. plasminogens (via the activation of kinin), immunoglobulins A and G, and complement proteins of the host (such as C3). In addition to their ability to degrade proteins of the host, they can also accelerate the process of inflammation, disabling thus host defense pathways. Over time, plaque becomes more complex, and its maturation in the subgingival environment is unchecked.¹⁸

Numerous microorganisms provide the products significant for the maintenance of others in a complex synergistic environment with the abundance of nutrients. Plaque microorganisms are a part of a complex microbial ecosystem, in which microbial diversity enables their own growth and reproduction.¹¹

LITERATURA / REFERENCES

1. Back JD, Koch GG, Zambon JJ, Genco RJ, Tudor GE. Evaluation of oral bacteria as risk indicators for periodontitis in older adults. *J Periodontol* 1992; 63: 93-99.
2. Barr CE. Oral diseases in HIV-I infection Dysphagia.1992; 7: 126-137.
3. Dzink JL, Gibbons RJ, Childs WC.III, Socransky SS. The predominant cultivable microbiota of crevicular epithelial cells. *Oral Microbiol. Immunol* 1989; 4: 1-5.
4. Grbić JT, Mitchell-Lewis DA, Fine JB, et al. The relationship of candidiasis to linear gingival erythema in HIV-infected homosexual men and parenteral drug user. *J Periodontol.* 1995; 66: 33-37.
5. Gregory RI, Kim DE, Kindle JC, Hobbs LC, Lloyd DR. Immunoglobulin-degrading enzymes in localized juvenile periodontitis. *J Periodont. Res.* 1992; 27: 176-183.
6. Grossi SG, Genco RJ, Machtei EE , et al. 1995; 66: 23-29.
7. Helgeland K, Nordby O. Cell cycle-specific growth inhibitory effect on human gingival fibroblasts of a toxin isolated from the culture medium of *Actinobacillus actinomycetemcomitans*. *J Periodont. Res.* 1993; 28: 161-165.
8. Kelly A, Antonio AG, Main LC, Luiz RR, Vianna RB. Reliability of plaque scoring index using photographs. *J Dent Res.* 2004; 83: Special Issue A.
9. Lotufo RF, Flynn J, Chen C, Slots J. Molecular detection of *Bacteroides forsythus* in human periodontitis. *Oral Micro. Immunol.* 1994; 9: 154-160.
10. Murray PA, Grassi M, Winkler JR. The microbiology of HIV-associated periodontal lesions. *J Clin Periodontol Res.* 1989; 6: 636-642.
11. Omar AA, Newman HN, Bulman J, Osborn J. Associations between subgingival plaque bacterial morphotypes and clinical indices. *J Clin Periodontol.* 1991; 18: 555-566.
12. Preber H, Bergstrom J, Linder LE. Occurrence of periopathogens in smokers and non-smokers patients. *J Clin Periodontol.* 1992; 19: 667-671.
13. Rams TE, Andriola M, Feik D, Abel SN, Me Given TM, Slots J. Microbiological study of HIV-related periodontitis. *J Periodontol.* 1991; 62: 74-81.
14. Reynolds MA, Minah GE, Peterson DE, et al. Periodontal disease and oral microbial successions during myelosuppressive cancer chemotherapy. *J Clin Periodontol.* 1989; 16: 185-189.
15. Schenkein HA, Best AM, Gunsolley JC. Influence of race and periodontal clinic status of neutrophil hemotactic response. *J Periodontol. Res.* 1991; 26: 272-275.
16. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease *J Periodontol.* 1992; 63: 322-331.
17. Tervonen T, Oliver RC, Wolff L, Bereuter J, Anderson L, Aeppli DM. Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. *J Clin Periodontol.* 1994; 21: 375-379.
18. Walker CB. Selected antimicrobial agents. Mechanisms of action, side effects and drug interactions. *Periodontol.* 2000. 1996; 10: 12-28.
19. Wolff LF, Aeppli DM, Pihlstrom B, Stoltenberg J. Natural distribution of 5 bacteria associated with periodontal disease. *J Clin Periodontol* 1993; 20: 699-706.
20. Zack AG, Contestable PB, Cottelear S, Snyder B. Longitudinal correlation of periodontal status with antigens from suspected periodontal pathogens. *J. Dent. Res.* 1993;72 (Spec. Issue):405 (Abstr. 2417)
21. Zambon JJ, Reynolds HS, Chen P, Genco RJ. Rapid identification of periodontal pathogens in subgingival dental plaque. Comparison of indirect immunofluorescence microscopy with bacterial culture for detection of *Bacteroides gingivalis*. *J Periodontol.* 1985; 56: 32-40.
22. Zambon JJ, Sunday JS, Smutko JS. Molecular genetic analysis of *Actinobacillus actinomycetemcomitans* epidemiology. *J Periodontol.* 1990; 61: 75-80.

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