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Utjecaj parodontološke terapije na C-reaktivni protein i parodontne patogene kod pacijenata s parodontopatijom

The Effects of Periodontal Therapy on C-reactive Protein and Periodontal Pathogens in Periodontitis Patients

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Sažetak
Nedavna epidemiološka istraživanja pokazuju da su osobe s parodontopatijom u mnogo većoj opasnosti od kardiovaskularnih bolesti. Iako mehanizam odgovoran za to potpuno jasan, pretpostavlja se da uklanjanje parodontne infekcije i inflamacije terapijom može smanjiti razinu upalnog markera CRP-a i rizik od koronarnih bolesti. **Svrha istraživanja**: Svrha ovog istraživanja bila je ustanoviti mogu li na razine CRP-a kod srbijanske populacije utjecati kronična parodontopatija i parodontni patogeni te kasnija terapija. **Materijali i metode**: Kod pedesetero ljudi s umjerenom do teškom parodontopatijom - a svi su obavili kompletan parodontalni debridment - pratilo se je li terapija parodontopatije smanjila upalu parodonta i snizila razinu upalnih markera u serumu. Sudionici su bili ispitani u vezi s težinom oboljelog parodonta, C-reaktivnog proteina i parodontnih patogena (*Porphyromonas gingivalis* i *Aggregatibacter actinomycetemcomitans*) tijekom prvog posjeta te šest i dvanaest mjeseci nakon terapije. U kontrolnoj skupini serum CRP-a uzet je od 25 ljudi bez parodontopatije. **Rezultati:** Razine CRP-a nisu bile niže od onih zabilježenih u zapadnim zemljama. Bilo je znatnih promjena u vrijednostima kliničkih parametara, razine CRP-a i parodontnih patogena prije završene terapije i nakon nje. **Zaključak:** Dobiveni rezultati upućuju na to da terapija može pridonijeti eliminaciji upale parodonta i parodontnih patogena te smanjiti razinu CRP-a u serumu. Parodontopatija može utjecati na upalne procese zbog toga što terapija parodonta smanjuje prisutnost parodontnih patogena i upalnih markera.

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Uvod

Parodontopatija je kronična infekcija prouzročena gramnegativnim bakterijama koje djeluju na potporna tkiva zuba (1). U mnogim studijama (2,3) ističe se činjenica da ona može biti novi modificirani čimbenik rizika kad je riječ o koronarnim bolestima. Iako se u istraživanjima proučavaju različite populacije i odabiru različita mjerenja za bolesti parodonta i kardiovaskularne bolesti, rezultati su dosljedni – u svima se ističe da parodontopatija može potaknuti koronarne procese (4,5,6).

Parodontopatija je lokalni upalni proces koji razara tkiva parodonta. No, ta se bolest očituje i upalnim odgovorom domaćina te ima sistemsku implikaciju kod zdravih ljudi i može djelomice pridonijeti povećanom riziku od kardiovaskularnih bolesti (KVO-a) kod pacijenata s parodontopatijom. Pretpostavlja se da inflamacija parodontne bolesti i rezultati produkcije cirkulirajućih citokina mogu pridonijeti razvoju ateroskleroze i kardiovaskularnih bolesti (2). Nedavni podaci iz literature upućuju na činjenicu da pacijenti s teškom parodontopatijom imaju povećanu razinu CRP-a, hiperfibrinogenemiju i umjerenu leukocitozu u odnosu prema

Introduction

Periodontitis is a chronic infection by predominantly Gram-negative bacteria that affects the supporting structures of the teeth (1). Many studies (2, 3) suggest that periodontal disease may be a novel, modifiable risk factor for coronary heart disease. Although these studies evaluated different populations and used different measuring criteria for periodontal and cardiovascular disease, the results are remarkably consistent, and the studies demonstrate that periodontal disease precedes coronary events (4, 5, 6).

Periodontitis is a local inflammatory process that leads to destruction of periodontal tissues. However, this disease is also characterized by systemic inflammatory host responses and, as such; it has systemic implication in healthy individuals and may partly contribute to a higher risk for cardiovascular disease (CVD) in patients with periodontitis. It has been hypothesized that the chronic inflammatory burden of periodontal disease and the resultant production of circulating cytokines may contribute to the development of atherosclerosis and cardiovascular disease (2). Recent evidence has indicated that patients with severe periodontitis have increased serum levels of

zdravoj kontrolnoj populaciji (7). Umjereno povećanje CRP-a upozorava na povećani rizik od kardiovaskularnih bolesti. Medijatori upale u sistemskoj cirkulaciji, kao što su CRP i fibrinogen, pokazatelji su općeg upalnog odgovora i ateroskleroze (8). Ta veza između upale i ateroskleroze upozorava na to da kronična infekcija, kao što je oralna kod parodontnih bolesti, može potaknuti kardiovaskularnu bolest. Na osnovi te tvrdnje u nekoliko je studija zabilježena povećana razina CRP-a kod pacijenata s parodontopatijom (9,10). Od tada se u mnogim istraživanjima ispituje veza između parodontopatije i KVO-a. S druge strane, rijetko koji autor izvještava da učinci parodontopatijske terapije smanjuju razinu serumskog CRP-a i utjecaj na sistemsko stanje (11). Kod pacijenata s parodontopatijom povišena razina CRP-a u serumu čak je povezana s visokom razinom infekcije parodontnim patogenima (12). Infektivni agensi mogu izravno potaknuti ozljede epitela i djelomice aktivirati upalni odgovor, kao kod ateroskleroze. Ima podataka da se infekcije prouzročene pojedinim bakterijama, kao što su *Chlamidia pneumoniae, Helicobacter pylori* i parodontni patogeni, javljaju zajedno sa srčanim bolestima. Parodontni patogeni mogu se sistemski širiti krvotokom i inficirati aterosklerotične plakove te izazvati upalu i nestabilnost (13). Četrdeset i četiri posto prisutnih ateroma sadržava barem jedan od parodontnih mikroorganizama (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*) (14).

U nedavnoj studiji *in vivo* zabilježena je bila pozitivna neovisna korelacija između infekcije parodonta i debljine karotidne intima-medije (15). Taj je odnos važan kod pacijenata inficiranih pretežno specifičnim bakterijama koje sudjeluju u etiologiji parodontopatije, uključujući *Porphyromonas gingivalis (P.g.), Aggregatibacter actinomycetemcomitans(A.a.) i Tannerella forsythia(T.f.)* (15).

U ovom istraživanju ispitivalo se (I) jesu li kod srbijanske populacije povećane razine CRP-a; (II) postoji li veza između CRP-a i G-negativnih bakterija i (III) odgovara li kod Srbijanaca stupanj individualnog odgovora na terapiju parodonta promjenama vrijednosti seroloških markera (CRP).

Materijal i metode

U ovom istraživanju sudjelovalo je pedesetero pacijenata s parodontopatijom - 23 žene i 27 muškaraca u dobi od 30 do 74 godine (prosječna dob 49,76 godina). U kontrolnoj skupini bilo je 25 ispitanika - 18 žena i 7 muškaraca u dobi od 30 do 65 godina (prosječna dob 43,52 godine). Nakon što su doznali svrhu istraživanja, svi su dali usmeni pristanak. Studija je bila provedena u skladu s Helsinškom deklaracijom i odobrio ju je Etički odbor Medicinskog fakulteta u Nišu (Br: 01-2800-5). Dobrovoljni ispitanici s neliječenom, teškom parodontopatijom bili su odabrani na Klinici za stomatologiju u Nišu. Kriteriji za sudjelovanje bili su: dob > 30 godina, umjerena do teška parodontopatija (najmanje četiri

CRP, hyperfibrinogenemia, moderate leukocitosis when compared with unaffected control population (7). Moderate elevation of C-reactive protein (CRP) has been found to be a predictor of increased risk for CVD. The levels of inflammatory mediators in the systemic circulation, such as C-reactive protein (CRP) and fibrinogen, are indicators of a general inflammatory response and atherosclerosis (8). This link between inflammation and atherosclerosis suggests that chronic infections, such as the oral infection in periodontal disease, may predispose to cardiovascular disease. Based on this paradigm, elevated CRP levels in periodontal patients have been reported by several studies (9,10). Since then, most studies have discussed the association between periodontal disease and CVD. Conversely, a small number of authors report that the effects of periodontal treatment decrease serum levels of CRP and its influence on systemic conditions (11). Furthermore, in periodontitis patients, elevated serum CRP levels are associated with high levels of infection with periodontal pathogens (12). Infectious agents may directly inflict injuries to the epithelium and partially activate the inflammatory response observed in atherosclerosis. There is evidence that infections with certain bacteria, such as *Chlamydia pneumonae*, *Helicobacter pylori* and periodontopathic bacteria are associated with heart disease. Periodontal pathogens may disseminate systemically through the blood stream and infect atherosclerotic plaques; they may cause inflammation and plaque instability (13). Forty-four percent of atheromas contained at least one of the periodontal microorganisms (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*) (14).

A recent study in humans reported a positive independent correlation between periodontal burden and carotid intima-media thickness (15). A positive relationship between carotid thickness and bacterial burden was especially strong in patients predominantly infected by specific bacteria involved in the etiology of periodontitis, including *Porphyromonas gingivalis (P.g.)*, *Aggregatibacter actinomycetemcomitans (A.a.)* and *Tannerella forsythia (T.f.)* (15).

In this study, we examined (i) whether CRP plasma levels are increased in Serbian periodontitis patients, (ii) relation between CRP and Gram-negative bacteria, and (iii) whether the degree of individual response to periodontal treatment was associated with changes in serological marker values of systemic inflammation (CRP) in a Serbian cohort.

Material and methods

A total of 50 patients with periodontitis (23 females and 27 males, age 30-74 years, mean age 49.76 years) participated in the study. There were 25 volunteers in the control group (7 males and 18 females, age 30-65, with mean age of 43.52). After being informed on the purpose of the study, all patients provided informed consent. The study was conducted in accordance with the Helsinki Declaration, and approved by Ethical Committee of the Faculty of Medicine, University of Niš (No:01-2800-5). Volunteer subjects with untreated, severe, advanced chronic periodontitis but otherwise in good general health, were recruited from the patient population of Dentistry Clinic, in Niš, Serbia. Criteria for inclusion were comprised

mjesta s dubinom džepa >5 milimetara nađenih u barem dva kvadranta i najmanje dva od četiri mjesta s gubitkom epitelnog pričvrstka >3 milimetra). Kriteriji za isključivanje bili su: bilo koja dentalna terapija u proteklih šest mjeseci, rak u povijesti bolesti, trudnoća, diabetes mellitus, kardiovaskularne bolesti ili terapija antihipertenzivima, antibioticima i nesteroidnim antireumaticima (NSAID).

Parodontna ispitivanja

Kliničke bolesti parodonta uključivale su krvarenje pri sondiranju (Ikrv), dubinu parodontnog džepa (DPDž) i gubitak pripojenog epitela (GPE). Parodontna mjerenja kod svakog su zuba bila obavljena na šest mjesta. Srednje vrijednosti dubine džepa i gubitka spojnog epitela izračunate su za svakog ispitanika kao srednja vrijednost svih mjerenih mjesta s dubinom džepa >5 milimetara i gubitkom epitelnog pričvrstka >3 milimetra.

Krvarenje pri sondiranju: ako krvarenje počne odmah nakon sondiranja dubine džepa, zabilježeno je kao pozitivno. Sondiranje parodontnog džepa mjerilo se standardnom parodontnom sondom (Michigan O). Gubitak spojnog epitela također se mjerio parodontnom sondom (Michigen O).

Sve varijable bile su procijenjene prije prvog tretmana te šest i dvanaest mjeseci nakon početka liječenja.

Ovo je istraživanje bilo eksperimentalno i u studiji su se ispitivali serum-marker sistemske upale (CRP) i prisutnost *Porphyromonas gingivalis* i *Aggregatibacter actinomycetemcomitans* i to prije terapije parodonta i nakon njezina završetka.

Na početku istraživanja ispitanici su pregledani i obavljeno je kompletno ispitivanje parodonta. Zatim je uzet uzorak venske krvi kako bi se analizirala razina CRP-a. Sudionici su nakon toga tijekom jednog ili dvaju posjeta bili uključeni u odgovarajuću terapiju. Kako bi se eliminirali parodontni patogeni i izbjegla ponovna infekcija, čišćenje i kiretaža svih parodontnih džepova te dezinfekcija cijele usne šupljine bila je završena vrlo brzo (u roku od 24 sata).

Ponovni pregled, uključujući pregled krvi i ispitivanje parodonta, bio je obavljen šest i dvanaest mjeseci nakon završetka terapije.

U sklopu prvog posjeta kod sudionika je bilo zabilježeno stanje oralne higijene i parametri upale parodonta. Na kraju su im dane upute kako trebaju održavati oralnu higijenu, predstavljena im je Bassova tehnika pranja zuba i upotreba interdentalnih četkica te im je pokazano kako se treba koristiti dentalnim koncem. Tretman je sadržavao kompletnu dezinfekciju usne šupljine koja je bila završena tijekom jednog ili dva posjeta u razdoblju od najmanje dva tjedna. Cjelokupni tretman obavio je jedan parodontolog. Sudionici su bili ponovno pozvani za dva tjedna. Kada su postigli dobru oralnu higijenu, obrada i kiretaža parodontnih džepova bila je obavljena pod lokalnom anestezijom. Nakon završene terapije, pacijenti su ponovno došli nakon šest te dvanaest mjeseci. Na tim pregledima ponovno su uzete vrijednosti parametara upale parodonta.

Uzorci krvi uzeti su na početku (prije tretmana) te šest i dvanaest mjeseci nakon njegova završetka. Testovi za određivanje razine serumskog CRP-a radili su se korištenjem radi-

of: age >30 years, moderate to severe chronic periodontal disease (at least 4 sites with pocket depth >5 mm distributed in at least 2 quadrants and at least 2 of the 4 sites with attachment loss >3 mm). Exclusion criteria were any dental treatment during the past 6 months and a medical history of cancer, pregnancy, Diabetes mellitus, CVD or treatment with antihypertensive medications, antibiotics and NSAID therapy.

Periodontal examination

Clinical periodontal measurements included bleeding on probing, periodontal pocket depth, and clinical attachment loss (CAL). Periodontal measurements were made on 6 sites of each tooth. Mean pocket depth and mean attachment level were calculated for each subject as the mean of all measured periodontal sites with pocket depth >5 mm and attachment level >3mm.

Bleeding on probing (BOP): If bleeding occurred immediately after probing for pocket depth, it was reported as positive. Probing pocket depth (PPD) was measured with a standard periodontal probe (Michigan O). Clinical attachment loss (CAL) was measured with a standard periodontal probe (Michigan O).

All periodontal variables were assessed before treatment as well as 6 and 12 months after periodontal treatment.

This study is a pilot trial in which serum markers of systemic inflammation (CRP) and the presence of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were measured before and after periodontal treatment.

At baseline, subjects were examined and a complete periodontal examination was performed, and then, a venous blood sample was taken for measurement of CRP. Subsequently, subjects received their indicated therapy over 1 or 2 visits. To eliminate periodontopathic bacteria and to avoid re-infection, scaling and root planing of all periodontal pockets was completed in a short period of time (within 24 hours) taking the full-mouth disinfection approach.

A post-treatment evaluation, which included a blood sample and periodontal examination, was conducted 6 and 12 months after the completion of periodontal therapy.

At the initial visit records of the patient's oral hygiene and of the periodontal variables were made. Oral hygiene instruction was given at the end of the visit. It included a demonstration of Bass brushing technique, a demonstration of the use of inter-dental brushes as well as a demonstration on the use of dental floss. Treatment was consistent with full-mouth disinfection and was completed over 1 or 2 visits occurring no less than 2 weeks apart. The entire periodontal treatment was performed by 1 periodontologist. Patients were recalled at two-week intervals. After the optimum oral hygiene had been obtained, periodontitis was treated with scaling and root planning which was performed under local anesthesia. Upon completion of the hygiene phase of the treatment, each patient was re-examined after 6 and after 12 months. During this visit, the periodontal variables detailed above were recorded again.

Blood samples were collected at baseline (before treatment) and 6 and 12 months after treatment. The levels of serum C-reactive protein assays were performed using radial

jalne imunodifuzije i bili su kvantificirani u mililitrima po litri. Razina C-reaktivnog proteina mjerila se na osnovi referentnih vrijednosti Glavnog biokemijskog laboratorija Kliničkog centra u Nišu.

Uzorci subgingivalnog plaka bili su uzeti od svakog ispitanika s najdubljeg mjesta parodontnog džepa, s dva zuba u svakom kvadrantu. Ima li parodontnih patogena (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans)* u uzorcima subgingivalnog plaka određivalo se PCR-metodom prije terapije i nakon što je završena. Detekcija bakterijske DNK u dentalnom plaku obavljala se multipleksnom PCR-tehnikom s različitim biološkim materijalima, što omogućuje istodobnu identifikaciju dvaju različitih bakterijskih genoma.

Statistička analiza

Svi podaci izraženi su kao srednja vrijednost i standardna devijacija (±SD), ili kao frekvencija i postotci. Razlike srednjih vrijednosti između skupina uspoređivale su se koristeći se ANOVA-analizom i post-hoc testom multiple komparacije. Chi-kvadrat test rabio se za neparametrijsko testiranje. Značajnost razlika između srednjih vrijednosti dobivenih prije terapije parodonta i nakon nje dokazani su Student t-testom za uzorke u paru. P-vrijednosti manje od 0,05 smatrale su se značajnima.

Rezultati

Osnovne demografske karakteristike ispitanika nalaze se u Tablici 1. koja pokazuje distribuciju, srednje vrijednosti, odd ratios i statističku značajnost socijalno-ekonomskih varijabla u ovom istraživanju.

Podaci su prikazani kao srednje vrijednosti (SD) ili kao postotci (%). Dob ispitanika bila je od 30 do 74 godine (prosjek 49,76±15,83 godina). Sudjelovalo je 46 posto žena i 55

immunodiffusion assay quantified in milligrams per liter.

The values of C-reactive protein were measured according to referent values from Central Biochemical Laboratory of Clinical Center in Niš.

We used a PCR Express Thermo Hybaid Model: HBPX 110 for microbiological testing. Subgingival plaque samples were obtained from curettage of the deepest subgingival sites of periodontal pockets from each patient, from two teeth in each quadrant. The presence of periodontal pathogens (*Porphyromonas gingivalis* and *Agregatibacter actinomycetemcomitans*) in subgingival plaque samples was measured by PCR method before and after periodontal therapy. Bacterial DNA detection was performed in diverse biological materials in dental plaque by means of multiplex PCR, a technique that allows simultaneous identification of two different bacterial genomes.

Statistical analysis

All data are created in the software program SPSS 10.0 and expressed either as mean ± standard deviation, or with frequencies and percents. Differences of mean values between groups were compared using ANOVA analysis and Post Hoc tests – multiple comparisons. Chi square test was used as a nonparametric test.

Differences between means obtained before and after periodontal treatment were examined for significance using the Student t-test for paired samples. P values below 0.05 were considered significant.

Results

Baseline demographic characteristics of the subjects are summarized in Table 1 which displays the distributions, means, crude odds ratios and statistical significances of the socio-economic variables in this study.

The data were presented as mean (SD) or percentage (%).The subjects' age ranged from 31-74, with a mean of 49.76±15.83 years, 46% were women and 55% men. The

Tablica 1. Osnovne karakteristike ispitanika
Table 1 Baseline characteristics of the study group

Varijable • Values	Ispitivana skupina • Study group (n=50)	Kontrolna skupina • Control group (n=25)	odds ratios	p vrijednost • p value
Spol • Sex, N°, %				
muški • male	27 (54.00%)	7 (28.00%)	3.02	0,0593
ženski • female	23 (46.00%)	18 (72.00%)	(0.96<OR<9.72)	ns
Godine, SV±SD • **Age**, mean±std	49.76±15.83	43.52±6.20		0,0173 <0,05
socijalna razina • Social level, N°, %				
niska • Low	33 (66.00%)	7 (28.00%)	4,99	0,0042
visoka, srednja • High, medium high	17 (34.00%)	18 (72.00%)	(1.56<OR<16.47)	<0,01
Godine školovanja • Years of education, N°,%				
<12 godina • <12years	32 (64.00%)	8 (32.00%)	3,78	0,0176
>12 godina • >12years	18 (36.00%)	17 (68.00%)	(1.22<OR<11.96)	<0,05
pušenje • Smoking, N°, %				
Da • Yes	19 (38.00%)	10 (40.00%)	0,92	0,9332
Ne • No	31 (62.00%)	15 (60.00%)	(0.31<OR<2.75)	ns

posto muškaraca. Razina obrazovanja bila je niska. Dvadeset devetero ispitanika (19 u ispitivanoj i 10 u kontrolnoj skupini) bili su pušači. Ni kod jednoga se tijekom istraživanja nije dogodila promjena statusa.

Bilo je više muškaraca u ispitivanoj skupini, ali ne statistički značajno više u odnosu prema kontrolnoj (p=0,0593). Osobe iz ispitivane skupine bile su znatno starije (p<0,05) i slabijeg socijalnog stanja (p<0,01) te niže razine izobrazbe - < 12 godina - (p<005). Nije bilo statistički značajne razlike između skupina kad je riječ o pušenju.

U Tablici 2. nalaze se kliničke karakteristike parodonta pacijenata s parodontopatijom na početku ispitivanja. Ispitivanja su pokazala statistički znatne vrijednosti krvarenja, dubine parodontnih džepova i gubitka spojnog epitela i CRP-a u odnosu prema različitim stupnjevima parodontopatije (p<0,001). Mjerenja su dala lošije rezultate za sve ispitivane varijable (krvarenje, dubina džepa i prisutnost *Porphyromonas gingivalis* i *Aggregatibacter actinomycetemcomitans* u odnosu prema kontrolnoj skupini).

Tablica 2. pokazuje vezu između parodontopatije i CRP-a, vrlo dobro poznatog čimbenika rizika kad je riječ o kardiovaskularnim bolestima. Statistički veliko smanjenje u razini CRP-a uočeno je kod ispitanika s oboljelim parodontom, u usporedbi sa zdravom kontrolnom skupinom (p<0,001).

Prema referentnim vrijednostima Glavnoga biokemijskog laboratorija Kliničkog centra u Nišu, prihvaćena razina CRP-a kao rizičnog čimbenika kod KVO-a jest >5 mg/l. Povišenje razine CRP-a bilo je znatno veće kod teške parodontopatije (9,58mg/l) u odnosu prema skupini s umjerenom parodontopatijom (5,19 mg/l) i onoj kontrolnoj (1,24 mg/l) (p<0,001).

Sudionici s velikom razinom gubitka spojnog epitela i dubljim parodontnim džepovima imali su statistički veće

socio-educational level was low. Twenty-nine patients (19 in the study group and 10 in the control group) were current smokers. No subjects changed their smoking status during the period of observation.

There were more men in the study group, but not statistically higher compared with the control group (p=0.0593). The subjects of the study group were statistically older (p<0.05) and were of lower social status (p<0.01) and lower status of education - <12 years – (p<0.05). There were no statistically significant differences between the groups regarding smoking.

Table 2 summarizes the clinical periodontal characteristics of the patients with periodontitis at baseline examination.

The examinations showed statistically significant values of BOP, PPD, CAL and CRP when compared to different stages of periodontitis (p<0.001).

The measurement showed worse results for all periodontal variables studied (BOP, pocket depth-PPD, and presence *Porphyromonas gingivalis* and *Aggreatibacter actinomycetemcomitans*) in comparison with the controls.

Table 2 exhibits the association between periodontitis and CRP, a well-known risk factor for cardiovascular disease. Statistically significant decreases in CRP levels were observed in subjects with periodontal disease when compared to healthy controls (p<0.001).

The reported range for CRP as a risk factor for CVD is >5 mg/l according to referent values from Central Biochemical Laboratory of Clinical Center in Niš. Elevated levels of CRP were significantly higher in the severe periodontitis group (9.58mg/l) when compared to moderate periodontitis group (5.19 mg/l) and the control group (1.24 mg/l) (p<0.001).

Tablica 2. Klinički parodontni parametri, razina C-reaktivnog proteina, prisutnost Porphyromonas gingivalis i Aggregatibacter actinomycetemcomitans kod ispitanika
Table 2. Clinical periodontal parameters, C-reactive protein levels, presence of P.g. and A.a. in the study participants

Varijable • Variable	Parodontopatija (PD) • Periodontitis (PD)		Kontrolna skupina • Control group (No=25)	p-vrijednost • p value
	Umjerena PD • Moderate PD (No=33) < 5mm	Teška PD • Severe PD (No=17) >5mm		
Ikrv# • BOP#	1.70±0.47 a***	1.88±0.33 a***	0.40±0.38	<0,001
DPDž§ • PPD§, mm				
SV±SD • Mean±Std	1,70±0,47 a***	1,88±0,33 a***	0,40±0,38	<0,001
GPE* • CAL*, mm				
SV±SD • Mean±Std	5,19±0,62 a***	6,74±0,60 ab***	1,97±0,54	<0,001
P.g.†, N°, %	18 (54.54%) a***	15 (88.24%) b**	0 (0.00%)	<0,001
A.a.‡, N°, %	11 (33.33%) a**	6 (35.29%) a***	0 (0.00%)	<0,01
CRPʃ, mg/l				
SV±SD • Mean±Std	5,19±2,95 a***	9,58±8,25 a**	1,24±1,46	<0,001

Legenda • Legend
[a] – u odnosu prema kontrolnoj skupini • vs control group; [b] – u odnosu prema umjerenoj • vs moderate
*** – p<0,001, ** – p<0,01 (Post hoc tests – multiple komparacije) • (Post Hoc Tests - Multiple Comparisons)
# **Ikrv** – indeks krvarenja • # **BOP** – bleeding on probing
§ **DPDž** – dubina parodontnog džepa • § **PPD** – periodontal pocket depth
* **GPE** – gubitak spojnog epitela • * **CAL** – clinical attachment loss
† **P.g.** – *Porphyromonas gingivalis* • † **P.g.** – *Porphyromonas gingivalis*
‡ **A.a.** – *Aggregatibacter actinomycetemcomitans*
ʃ **CRP** – C-reaktivni protein • C reactive protein

srednje vrijednosti CRP-a (9,58±8,25 mg/l) negoli kontrolna skupina (1,24±1,46mg/l), (p<0,001).

Prisutnost parodontnih patogena *Porphyromonas gingivalis* (p<0,001) i *Aggregatibacter actinomycetemcomitans* (p<0,01) u subgingivalnom plaku bila je pozitivno udružena s težinom bolesti parodonta.

Učinci terapije na parodontne varijable i razina CRP-a u plazmi prikazani su u Tablici 3.

Nakon terapije popravile su se parodontne varijable u odnosu prema mjerenjima na početku ispitivanja. Indeks krvarenja bio je znatno bolji. Prije terapije iznosio je 1,76 u odnosu prema 0,42 nakon terapije (p<0,001). Nakon terapije bila je poboljšana i dubina džepa – od 4,68 mm smanjena je na 2,97 mm (p<0,001). Vrijednosti gubitka spojnog epitela pokazale su slično poboljšanje – od 5,71 na 4,11 (p<0,001). Razine markera (biljega) upale bile su različite na početku tretmana i nakon njegova završetka. Nakon tretmana srednja se vrijednost CRP-a smanjila u odnosu prema vrijednosti na početku istraživanja sa 6,69mg/l na 4,25mg/l. Uspoređivanje bakterija odnosi se na njihovo smanjenje. *Porphyromonas gingivalis* bio je prije tretmana zastupljen sa 66 posto, 22 posto nakon terapije (poslije šest mjeseci) i 8 posto nakon dvanaest mjeseci (p<0,001). Razina *Aggregatibacter actinomycetemcomitans* prije terapije iznosila je 34 posto, a nakon toga 12 posto i 6 posto (p<0,01).

Nakon terapije statistički su se značajno smanjile srednje vrijednosti svih ispitivanih parodontnih parametara (p<0,001) u ispitivanoj skupini. Također su se statistički značajno smanjili parodontni patogeni *Porphyromonas gingivalis* (p<0,001) i *Aggregatibacter actinomycetemcomitans* (p<0,01).

Subjects with high values of mean clinical attachment loss and deeper periodontal pocket had significantly higher mean CRP levels (9,58±8,25 mg/l) than controls (1,24±1,46mg/l), (p<0.001).

The presence of periodontal pathogens *Porphyromonas gingivalis* (p<0.001) and *Aggregatibacter actinomycetemcomitans* (p<0.01) in subgingival samples was positively associated with severity of periodontal disease.

The effects of the periodontal treatment on the periodontal variables and the plasma levels of CRP are indicated in Table 3.

Tablica 3. Parodontne varijable razina CRP-a u plazmi (SV±SD) prije terapije parodonta i nakon njezina završetka
Table 3 Dental variables and plasma levels of CRP (mean±SD) before and after periodontal treatment

Varijable • Variable	Na početku • Baseline	Nakon terapije • After treatment		p-vrijednost • p value
		6 mjeseci • 6 months	12 mjeseci • 12 months	
Ikrv[#] • BOP[#]				
SV±SD • mean±Std	1.76±0.43	0.42±0.46	0.42±0.37	p<0.001
DPDž[§] • PPD[§], mm				
SV±SD • mean±Std	4.68±1.11	3.13±0.57	2.97±0.53	p<0.001
GPE[*] • CAL[*], mm				
SV±SD • mean±Std	5.71±0.96	4.27±0.57	4.11±0.53	p<0.001
CRP[ʃ], mg/l				
SV±SD • mean±Std	6.69±5.69	4.94±4.21	4.25±2.63	p<0.001
P.g.[†], N°,%	33 (66.00%)	11 (22.00%)	4 (8.00%)	p<0.001
A.a.[‡], N°,%	17 (34.00%)	6 (12.00%)	3 (6.00%)	p<0,01

Legenda • Legend:
[#] **Ikrv** – indeks krvarenja • [#] **BOP** – bleeding on probing
[§] **DPDž** – dubina parodontnog džepa • [§] **PPD** – periodontal pocket depth
[*] **GPE** – gubitak spojnog epitela • [*] **CAL** – clinical attachment loss
[†] **P.g.** – *Porphyromonas gingivalis*
[‡] **A.a.** – *Aggregatibacter actinomycetemcomitans*
[ʃ] **CRP** – C-reaktivni protein • [ʃ] **CRP** – C reactive protein

After periodontal therapy, periodontal variables improved compared to the baseline values; measurements of BOP showed significant improvements. Before therapy, it was 1.76 compared to 0.42 after the therapy (p<0.001). After therapy, there were significant improvements in the pocket depth - from 4.68mm to 2.97mm (p<0.001). Values of CAL showed similar improvements – from 5.71 to 4.11 (p<0.001). Levels of inflammatory marker at baseline and after treatment were different. After treatment, median serum of CRP decreased from the baseline value of 6.69mg/l to 4.25mg/l. Comparison of bacteria revealed a reduction in periodontal pathogens. The presence of *Porphyromonas gingivalis* was 66% before treatment, 22% after the treatment (at 6 months) and 8% at 12 months (p<0.001). The level of *Aggregatibacter actinomycetemcomitans* before treatment was 34% and after the therapy it was lower (12% and 6%), (p<0.01).

After the therapy, there was a statistically significant decrease of mean values of all examined periodontal clinical parameters (p<0.001 in the study group. There were statistically significant decreases of periodontal pathogens – *Porphyromonas gingivalis* (p<0.001) and *Aggregatibacter actinomycetemcomitans* (p<0.01).

Rasprava

Ova eksperimentalna studija rezultirala je važnim podacima te upozorava na činjenicu da uklanjanje infekcije parodonta terapijom poboljšava zdravlje parodonta i smanjuje razinu medijatora akutne faze upalnog odgovora. Istaknuto je također da osnovni nekirurški tretman parodonta kod pacijenata s umjerenom i teškom parodontopatijom, klinički i statistički znatno poboljšava parodontni status, redukciju razine subgingivalnih bakterija te smanjuje razinu CRP-a u serumu. Razina CRP-a u serumu znatno je smanjena kod pacijenata s parodontopatijom (p<0,001). Nakon tretmana, razine CRP-a u serumu smanjuju se (4,94mg/l i 4.25mg/l) i ta je razlika statistički značajna (p<0,001).

U nekoliko studija ispitivala se veza između bolesti parodonta i razine CRP-a (16,17). Osobe s bolesnim parodontom imaju više razine CRP-a, nego sudionici u kontrolnoj skupini. Pacijenti s teškom parodontopatijom pokazivali su jaču povezanost s kardiovaskularnim bolestima. Ti ljudi također imaju veću količinu patogenih bakterija i sistemskih čimbenika koji mogu pridonijeti izmijenjenom imunosno-upalnom odgovoru. Važan dokaz je i identificirana ateroskleroza kao upalna bolest. Pojedinci mogu imati genetsku predispoziciju za hiperinflamatorni odgovor na bakterijski izazov, kao što je bolest parodonta.

Rezultati iz ove studije pokazali su da je u srbijanskoj populaciji razina CRP-a bila viša kod ljudi s parodontopatijom negoli u kontrolnoj skupini, što je u skladu s dosadašnjim istraživanjima (10,18). Dakle, naši rezultati da se upalni markeri smanjuju tijekom terapije slažu se s nalazima D'Aiuta i suradnika (19) koji su mjerili serumske upalne markere kod 24 pacijenta prije nekirurške terapije teške generalizirane parodontopatije i nakon njezina završetka. U usporedbi s početnim vrijednostima, već nakon šest mjeseci bilo je uočeno znatno smanjenje razine CRP-a. U našoj se studiji smanjenje vrijednosti CRP-a dogodilo nakon dvanaest mjeseci. Ta se razlika može objasniti činjenicom da smo nakon terapije dvanaest mjeseci održavali zdravlje parodonta. Nakon terapije bilo je zabilježeno i veliko poboljšanje u postotcima, kad je riječ o prisutnosti parodontnih patogena.

U ovom istraživanju potvrđeno je da parodontopatijska terapija smanjuje razinu CRP-a i parodontnih patogena, ali i da se stanje može poboljšati bez primjene sistemske terapije antibioticima, što upućuje da je poboljšanje rezultat lokalne intervencije.

Pozitivna korelacija između CRP-a i parodontopatije najvjerojatnije je osnovna veza između bolesti parodonta i analiziranog većeg rizika od KVO-a kod tih pacijenata, zato što je CRP marker kardiovaskularnog rizika.

Upala desni kod parodontopatije može na različite načine utjecati na aterosklerozu. Oralna infekcija parodontnim patogenima počinje akumulacijom dentalnog biofilma te završava upalom tkiva parodonta. Nastanak parodontnih džepova povećava subgingivalni prostor te on postaje pogodan za depozit i razvoj bakterija. Lokalni upalni procesi koji slijede potiču mikroulceraciju u epitelu džepa te stvaraju rizik od infekcija na udaljenim mjestima i tranzitne bakterijemije (20). Osim toga, bakterije oslobađaju različite biološki ak-

Discussion

This pilot study provides important evidence that a near-complete elimination of periodontal infection by local periodontal therapy can result in better periodontal health and in decreased levels of mediators of the acute-phase inflammatory response. The present study demonstrates that basic nonsurgical periodontal treatment in patients with moderate to severe periodontitis results in clinically and statistically significant improvements in periodontal status, reduction of subgingival bacterial levels leading to a significant suppression of serum C-reactive protein. Serum CRP levels were significantly decreased in patients with periodontitis (p<0.001). After treatment, serum CRP levels decreased (4.94mg/l and 4.25mg/l) and the differences were statistically significant (p<0.001).

Few previous studies have evaluated the relationship between periodontal disease and levels of CRP (16,17). Subjects with periodontal disease had higher CRP levels than controls. People with severe periodontitis have the strongest link to cardiovascular disease. These people also have the greatest amounts of pathogenic bacteria and systemic factors that may contribute to an altered immunoinflammatory response. Considerable evidence has identified atherosclerosis as an inflammatory disease. Certain people may be genetically predisposed to experience a hyperinflammatory response when stimulated by a bacterial challenge such as periodontal disease.

The results of this study on Serbian population showed that higher CRP levels were found in subjects with periodontal disease than in controls, which is consistent with similar previous studies (10, 18). Therefore, our observation that serum inflammatory markers are decreased by periodontal therapy is consistent with the findings of D'Aiuto, et al.(19) who measured serum inflammatory markers in 94 subjects before and after nonsurgical therapy for severe generalized periodontitis. Compared with baseline values, a significant decrease in CRP was evident only at 6 months. In our study, a decrease in CRP was at 12 months. This difference may be explained by the fact that we maintained periodontal health after therapy at 12 months. After therapy, there were significant improvements in percentage of the presence of periodontal pathogens.

This study confirms that treatment of periodontal disease leads to decreased CRP and presence of periodontal pathogens, and it also demonstrates that improvement is possible in the absence of systemic antibiotic therapy, suggesting that it is the result of a localized intervention.

The positive correlation between CRP and periodontal disease might be a possible underlying pathway in the association between periodontal disease and the observed higher risk for CVD in these patients because CRP is a marker of cardiovascular risk.

Gingival inflammation in periodontitis may influence atherosclerosis in distinct pathways. Oral infection by periodontal pathogens initiates the formation of dental biofilm which leads to inflammation of periodontal tissues. The formation of periodontal pocket increases subgingival space

tivne molekule, uključujući i lipopolisaharide koji mogu ući u sistemsku cirkulaciju. Ti produkti mogu potaknuti upalni odgovor i u serumu povećati koncentraciju reaktanata akutne faze i medijatora upale, kao što je CRP. Smatra se da povećana razina cirkulirajućih medijatora upale može pridonijeti nastanku upalnih procesa, što vodi prema nastanku ateroskleroze (21).

Tradicionalni markeri kardiovaskularnog rizika kao što su godine, hiperlipidemija, hipertenzija, diabetes mellitus i pušenje, te novi čimbenici rizika, tj. upala, javljaju se zajedno s kardiovaskularnim bolestima.

Oralna upala i bolesti parodonta uglavnom su kronična stanja i mogu godinama asimptomatski perzistirati bez odgovarajuće terapije. Posljedica te kronične izloženosti na lokalnu sistemsku upalu može potaknuti ili poboljšati već postojeću upalnu bolest, uključujući i aterosklerozu. Modifikacija tih rizičnih čimbenika zauzvrat potiče poboljšanje zdravlja parodonta i zato može biti dobar pokazatelj za evaluaciju novih strategija kako bi se smanjio rizik. Zato je odgovarajuća preventiva oralne higijene važna ne samo da bi se sačuvalo oralno zdravlje, nego i radi poboljšanja sistemskog zdravlja. Promotori stomatološke zaštite i pacijenti moraju biti obaviješteni o preventivnim mjerama i važnosti dobrog oralnog zdravlja za sistemske poteškoće i kronične bolesti.

Rana kontrola akumulacije bakterijskog biofilma, zajedno s dnevnim mehaničkim uklanjanjem bakterijskog plaka četkicom i interdentalnim čišćenjem, važna je radi prevencije oralne upale i bolesti parodonta.

Bolesti parodonta i ateroskleroza imaju važne rizične čimbenike, kao što su pušenje, diabetes mellitus i pretilost te zbog toga vjerojatno i koegzistiraju kod mnogih pacijenata. Oni kod kojih se javlja izraženi upalni odgovor na čimbenike okoliša mogu biti predisponirani za razvoj vaskularnih lezija i kod parodontopatije i kod ateroskleroze. Naši rezultati pokazuju da terapija parodontopatije poboljšava zdravlje parodonta i smanjuje razinu CRP-a, te su u skladu s mogućom ulogom parodontopatije u patogenezi ateroskleroze, možda posredno preko sistemske upale. Modifikacija tih rizičnih čimbenika potiče razvoj te bolesti (22).

Smatra se da je C-reaktivni protein glavni reaktant akutne faze upale. Ako mu je razina povećana, to je snažan čimbenik rizika kad je riječ o koronarnim bolestima, moždanom udaru i bolestima perifernih arterija (23). U ovom je istraživanju razina CRP-a u serumu bila niža nakon terapije. Ti se nalazi slažu s onima u ostalim studijama (24). Treba napomenuti da je redukcija CRP-a u serumu bila veća kod intenzivne terapije parodonta (primjena sistemske ili lokalne antibiotske terapije uz standardnu parodontopatijsku terapiju) u odnosu prema primjeni samo standardne terapije (25,26).

Iako se u dosadašnjim studijama upozorava na vezu između parodontopatije i kardiovaskularnih bolesti, objašnjenje još ne zadovoljava. U našem ispitivanju nekoliko je važnih ograničenja. Zbog malog uzorka, naše istraživanje je eksperimentalno i bilo bi ga teško uklopiti u neku veću i detaljniju analizu. Na kraju – u ovo istraživanje nije bila uključena kontrolna skupina neliječenih pacijenata. No, razdoblje praćenja od dvanaest mjeseci dovoljno je da pokaže poboljšanja nakon što je uklonjena infekcija (manje parodontnih pa-

which is conductive for bacterial growth and deposits. Ensuing local inflammation processes produce micro-ulceration through the pocket epithelium, promoting risks for distant-site infection and transient bacteriemia (20). Moreover, bacteria release a variety of biologically active molecules, including lipopolysaccharides that may then enter the systemic circulation. These products can trigger the host inflammatory response and elevate serum concentration of acute-phase reactants and inflammatory mediators such as CRP. Increased levels of circulating inflammatory mediators are thought to contribute to the inflammatory processes leading to atherosclerosis (21).

Traditional markers of cardiovascular risk such as age, hyperlipidemia, hypertension, diabetes, lifestyle and tobacco use, as well as novel risk factors such as inflammation are associated with cardiovascular disease.

Oral inflammation and periodontal disease are generally chronic and can persist asymptomatically for many years in the absence of appropriate treatment. The consequence is a chronic exposure to local and systemic inflammation, which may induce or enhance already existing inflammatory disease, including atherosclerosis. Modification of these risk factors, in turn, induces improvement in periodontal health and therefore, may be a promising index for evaluating novel strategies of risk reduction. For this reason, appropriate oral preventive care is important not only to preserve oral health, but also to enhance systemic health. Management and prevention strategies must sensitize both dental care providers and patients about the importance of good oral health to the systemic burden and chronic diseases.

Early control of bacterial biofilm accumulation is essential for the prevention of oral inflammation and periodontal disease. Daily mechanical removal of bacterial plaque by tooth brushing supplemented with interdental cleaning is also vital.

Periodontal disease and atherosclerosis share important risk factors, such as tobacco use, diabetes, and obesity, and are therefore likely to coexist in many patients. Alternatively, individuals who exhibit a pronounced inflammatory response to environmental influences may be predisposed to the development of both periodontal disease and atherosclerotic vascular lesion. Our finding that periodontal therapy improves periodontal health and decreases CRP levels is consistent with a possible causative role for periodontal disease in the pathogenesis of atherosclerosis, perhaps mediated through systemic inflammation. Modification of these risk factors induces improvement of this disease (22).

CRP is considered to be a major acute-phase reactant in humans. Elevated CRP level is a strong determinant of coronary heart disease risk, stroke and peripheral arterial disease (23). In the present study, serum CRP levels were lower after treatment. These findings are in agreement with some other studies (24). It is of note that the reduction in serum CRP was greater in intensive periodontal treatment (systemic or local antibiotics with standard periodontal treatment) compared with standard periodontal treatment alone (25,26).

Although observational studies suggest an association between periodontitis and cardiovascular disease, the explanation for this relationship is not fully understood. Our study

togena *Porphyromonas gingivalis* i *Aggregatibacter actinomycetemcomitans* i bolje vrijednosti parodontnih parametara) te smanjenje razine CRP-a. Promjene drugih, neprepoznatljivih čimbenika koje nismo ispitivali, mogle su pridonijeti postignutom poboljšanju u razdoblju praćenja.

Zaključak

Ovom se studijom pokazalo da smanjenje razine CRP-a kod pacijenata s parodontopatijom ovisi o težini bolesti i prisutnosti parodontnih patogena. Povećana razina CRP-a također je bila češća ako je istodobno bila zabilježena i subgingivalna infekcija u sklopu bolesti parodonta.

Ovo istraživanje također upućuje na činjenicu da terapija bolesti parodonta smanjuje markere upale i poboljšava zdravlje parodonta.

Kontrola infekcije i upale parodonta poboljšat će oralno zdravlje, smanjiti djelovanje kronične sistemske infekcije prouzročene oralnom infekcijom, poboljšati opće zdravlje i na kraju smanjiti nastanak kardiovaskularnih bolesti.

has several important limitations. It has been a pilot study due to the small sample size and would be difficult to incorporate into a larger, more definite trial. Finally, our observational pilot study did not include a control group of untreated patients. But the follow-up period of 12 months is enough to show improvements after elimination of infection (less presence of periodontal pathogens – *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, and improvement in periodontal parameters values) and decrease of CRP levels. Changes in other unrecognized factors may have contributed to the observed improvements in the follow-up period, which we have not evaluated.

Conclusion

Our study demonstrated that the extent of decrease in CRP levels in periodontitis patients depends on the severity of the disease and present periodontal pathogens. Also, the elevated levels of CRP are more often associated with subgingival infection related to periodontal disease.

Our pilot trial suggested that treatment of periodontal disease may lead to a decrease in inflammatory markers and improvement of periodontal health.

Control of periodontal infection and inflammation will improve the oral health of patients, decrease the systemic chronic inflammation burden caused by oral infection, improve general health, and may ultimately contribute to the reduction of cardiovascular disease.

Abstract

Objective. Recent epidemiological studies have shown that individuals with periodontitis have significantly increased risk of developing coronary heart disease. Although the mechanism responsible for the relationship between periodontal disease and cardiovascular events is not fully understood, it is hypothesized that the removal of infection and inflammatory burden of periodontitis by therapy would be accompanied by a decrease in levels of the inflammatory marker C-reactive protein and a decreased risk of coronary heart disease. The aim of the present study was to determine whether the presence of chronic periodontitis and periodontal pathogens and subsequent periodontal treatment could influence the serum levels of C-reactive protein in a Serbian cohort. **Material and Methods:** Fifty adults with moderate to severe periodontitis who underwent complete mouth disinfection were evaluated to determine if periodontal therapy would result in decreased periodontal inflammation and lower serum levels of an inflammatory marker. Subjects underwent measuring of periodontal disease severity and serum C-reactive protein, and periodontal pathogens (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) at the time of the baseline visit and again 6 and 12 months after treatment. Serum levels CRP were also obtained from 25 subjects without periodontitis in a control group. **Results:** The levels of CRP in the serum seemed not to be lower than those reported in other population in Western European countries. There were significant changes in clinical periodontal values, CRP levels and presence of periodontal pathogens when baseline values were compared to those taken after periodontal treatment completion. **Conclusion:** The obtained results confirm the hypothesis that periodontal therapy may contribute to elimination of periodontal inflammation and periodontal pathogens, and reduce serum level of CRP. Periodontitis may contribute to the systemic inflammatory burden of affected individuals since it was shown that periodontal therapy may decrease presence of periodontal pathogens and inflammatory markers.

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References

1. Carranza FA, Newman MG, Takei HH. Carranza's Clinical Periodontology. Philadelphia: WB Saunders; 2002.
2. Beck JD, Offenbacher S, Williams R, Gibbs P, Garcia R. Periodontitis: a risk factor for coronary heart disease? Ann Periodontol. 1998 Jul;3(1):127-41.
3. Joshipura KJ, Hung HC, Rimm EB, Willett WC, Ascherio A. Periodontal disease, tooth loss, and incidence of ischemic stroke. Stroke. 2003 Jan;34(1):47-52.
4. Hung HC, Willett W, Merchant A, Rosner BA, Ascherio A, Joshipura KJ. Oral health and peripheral arterial disease. Circulation. 2003 Mar 4;107(8):1152-7.
5. Jansson L, Lavstedt S, Frithiof L, Theobald H. Relationship between oral health and mortality in cardiovascular diseases. J Clin Periodontol. 2001 Aug;28(8):762-8.
6. Tuominen R, Reunanen A, Paunio M, Paunio I, Aromaa A. Oral health indicators poorly predict coronary heart disease deaths. J

Dent Res. 2003 Sep;82(9):713-8.
7. Wu T, Trevisan M, Genco RJ, Falkner KL, Dorn JP, Sempos CT. Examination of the relation between periodontal health status and cardiovascular risk factors: serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. Am J Epidemiol. 2000 Feb 1;151(3):273-82.
8. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999 Jan 14;340(2):115-26.
9. Ajwani S, Mattila KJ, Närhi TO, Tilvis RS, Ainamo A. Oral health status, C-reactive protein and mortality--a 10 year follow-up study. Gerodontology. 2003 Jul;20(1):32-40.
10. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol. 2008 Apr;35(4):277-90.
11. Mattila K, Vesanen M, Valtonen V, Nieminen M, Palosuo T, Rasi V et al. Effect of treating periodontitis on C-reactive protein levels: a pilot study. BMC Infect Dis. 2002 Dec 10;2:30.
12. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol. 2001 Sep;72(9):1221-7.
13. Herzberg MC, Weyer MW. Dental plaque, platelets, and cardiovascular diseases. Ann Periodontol. 1998 Jul;3(1):151-60.
14. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol. 2000 Oct;71(10):1554-60.
15. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR Jr, Sacco RL et al. Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). Circulation. 2005 Feb 8;111(5):576-82.
16. Loos BG. Systemic markers of inflammation in periodontitis. J Periodontol. 2005 Nov;76(11 Suppl):2106-15.
17. Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. Arch Intern Med. 2003 May 26;163(10):1172-9.
18. D'Aiuto F, Parkar M, Tonetti MS. Acute effects of periodontal therapy on bio-markers of vascular health. J Clin Periodontol. 2007 Feb;34(2):124-9.
19. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. J Periodontal Res. 2004 Aug;39(4):236-41.
20. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135-87.
21. Scannapieco FA. Periodontal inflammation: from gingivitis to systemic disease? Compend Contin Educ Dent. 2004 Jul;25(7 Suppl 1):16-25.
22. Blumenthal JA, Sherwood A, Babyak MA, Watkins LL, Waugh R, Georgiades A et al. Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease: a randomized controlled trial. JAMA. 2005 Apr 6;293(13):1626-34.
23. Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? J Hypertens. 2003 Oct;21(10):1787-803.
24. Singer RE, Biesbock AR, Cavanaugh PF, Nibali L. Relationships of systemic biomarkers to oral hygiene habits and practices. Ann Periodontol. 2002;7(1):121-2.
25. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J Dent Res. 2005 Mar;84(3):269-73.
26. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J. 2006 May;151(5):977-84.