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Razina upalnih medijatora u slini pacijenata s parodontitisom: pilot studija

Whole Saliva Levels of Some Inflammatory Mediators in Patients with Previous Evidence of Periodontitis: a Pilot Study

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Sažetak
Svrha: Upalni medijatori čine skupinu proteina aktivno uključenih u upalni proces, no malo se zna kolike su njihove razine u slini pacijenata s parodontitisom. **Ispitanici i postupci:** Kako bismo ustanovili njihovu količinu i koncentraciju, izmjerili smo razine alkalne fosfataze (ALP-a), laktoferina (LF-a), mijeloperoksidaze (MPO-a), interleukina-1 (IL-1), beta-2 mikroglobulina (β_2-MG) te GRO-α kod pacijenata oboljelih od parodontitisa na početku parodontalne terapije te nakon inicijalnog liječenja. Uzorci sline 16 ispitanika s kliničkim znakovima i simptomima parodontitisa prikupljani su na početku terapije te šest tjedana nakon nje. **Rezultati:** Statistička analiza otkrila je nakon terapije u ispitnoj skupini velike razlike u vrijednostima kliničkih parodontalnih parametara te u razini kemokina. Koncentracije CRP-a, LF-a, GRO-a, MPO-a, IL-1 i ALP-a bile su nakon cijeljenja snižene, a povećala se koncentracija β_2-MG-a. **Zaključak:** Može se zaključiti da su koncentracije citokina u slini mogući indikatori rizika za aktivnu parodontalnu bolest.

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Ključne riječi
periodontitis; slina; alkalna fosfotaza; laktoferin; peroksidaza; interleukin-1; ELISA; citokini

Uvod

Prisutnost i diferencijacija stanica u upalnom području ovisi o kemotaktičkim agensima koji se stvaraju lokalno. Velika superobitelj tih citokina („kemokina“) danas se sastoji od više od 40 molekula

Introduction

The presence and recruitment of cells into an inflammatory area depends on chemotactic agents that form locally. A great superfamily of chemotactic cytokines (“chemokines”) consists today of more than

(1). Kemokini su topljivi proteini i djeluju kao kemoatraktanti, najviše na leukocite kao najjače aktivatore imunokompetentnih stanica.

Iako još nije poznata njihova fiziološka zadaća u homeostazi, njihova uloga nadmašuje samu kemoatrakciju. Mehanizam na ciljnim stanicama uglavnom potiču medijatori, a aktivnost receptora ograničena je na određene klase kemokina koji definiraju određene biološke učinke. Tako α-kemokini uglavnom djeluju na neutrofile, a manje na fibroblaste. Ta podobitelj uključena je u upalne procese koje potiču neutrofili. Uglavnom na monocite djeluju β-kemokini, ali zna se da djeluju i na bazofile, eozinofile, limfocite, astrocite, dendritičke stanice, fibroblaste i hematopoietske progenitorske stanice (1).

Opće je prihvaćeno mišljenje da je odgovor domaćina presudna karika u upalnoj parodontalnoj bolesti (2). Koncentracija neutrofila, makrofaga i limfocita u oštećenom ili upaljenom području vrlo je važna u obrani od nametnika. Među svim tim stanicama neutrofili su najvažniji, budući da stvaraju prvu crtu obrane. Što ih je manje, to je progresija bolesti brža, a destrukcija veća. Neutrofili sadržavaju tvari potrebne za fagocitozu i uništavanje patogenih mikroorganizama - laktoferin (LF, μg/l), alkalnu fosfatazu (ALP, U/l), kisele hidrolaze, kationske proteine, stimulirajući čimbenik rasta melanoma (GRO/MGSF, ng/ml) te mijeloperoksidazu (MPO, U/mL). Mehanizmi djelovanja tih molekula dobro su poznati (3).

Interleukin-1 (IL-1), ključni regulator destrukcije tkiva i čimbenik nekroze tumora-α (TNF-α) može inducirati proizvodnju matriksnih metaloproteinaza koje uključuju kolagenazu (MMP-1), gelatinazu (MMP-2) te stromelizin (MMP-3). IL-1β može uzrokovati resorpciju kosti i pojačati osteoklastičku aktivnost. Sinteza i lučenje određenih medijatora iz mononuklearnih fagocita mogu smanjiti stupanj destrukcije (4). Kabashima i suradnici (5), ali i drugi autori, izolirali su IL-1 na mjestima s kroničnim parodontitisom (6,7). Dokazano je da to može biti rani pokazatelj-marker za gingivitis, a javlja se prije kliničkih znakova upale. IL-1 djeluje na makrofage, monocite, T- i B-limfocite, osteoklaste, fibroblaste, endotelne stanice te keratinocite.

Vrlo se malo zna o GRO-u, iako je potvrđeno njegovo djelovanje u kemotaksiji neutrofila i razvoju upale. U kulturama humanih gingivalnih fibroblasta dokazano je da ga inducira IL-1β (8), a proizvode ga makrofagi, monociti, neutrofili, fibroblasti, epitelne i endotelne stanice, hondrociti te osteociti. C-reaktivni protein (CRP, mg/l) smatra se mogućim

40 molecules (1). Chemokines are soluble proteins that act both as chemoattractants, having the most effect on leukocytes, being the strongest activators of these immunocompetent cells. Although their physiologic role in the homeostasis is not yet determined, their influence encompasses mere chemoattraction. The mechanism on target cells is mainly receptor-mediated, the receptor cross-activity being restricted for a certain chemokine class that defines different biological effects. α-chemokines act mainly on neutrophils and to a smaller extent to fibroblasts. This subfamily is included in neutrophil-mediated inflammatory processes. β-chemokines act mainly on monocytes, but their effect on basophiles, eosinophiles, lymphocytes, astrocytes, dendritic cells, fibroblasts and haematopoietic progenitor cells is known as well (1).

It has been widely accepted that the host response represents the decisive link in the inflammatory periodontal disease (2). Concentration of neutrophils, macrophages and lymphocytes in the damaged or inflamed areas is one of the most important steps in the defense against the intruders. Among these cells, neutrophils play the most important role, since they establish the first line of defense. The smaller their number, and/or their weakened function, the more rapid progression of the disease and destruction of the periodontal health can be expected. Neutrophils contain the material necessary for phagocytosis and killing of the pathogenic microorganisms, lactoferrin (LF, μg/l), alkaline phosphatase (ALP, U/l), acid hydrolases, cationic proteins, melanome growth stimulating factor (GRO/MGSF, ng/ml) and myeloperoxidase (MPO, U/mL) among others. Mechanisms of these molecules are well known (3).

Interleukin-1 (IL-1, μg/l), the key regulator of tissue destruction, and tumor necrosis factor-α (TNF-α), can induce the production of a group of matrix metalloproteinases that include collagenase (MMP-1), gelatinase (MMP-2) and stromelysin (MMP-3). IL-1β can cause bone resorption and can increase osteoclastic activity. The synthesis and secretion of certain mediators in mononuclear phagocytes can diminish the rate of tissue destruction (4). Kabashima et al. (5) have isolated IL-1 in sites with chronic periodontitis, as well as other authors (6,7). It has been proven that IL-1 can be an early marker of gingivitis that precedes clinical signs of inflammation. IL-1 acts on macrophages, monocytes, T- and B-lymphocytes, osteoclasts, fibroblasts, endothelial cells and keratinocytes. Very little is known

medijatorom povezanosti parodontitisa i nekoliko sistemskih bolesti, a nalazimo ga u aktivnim parodontalnim lezijama (9). β2-mikroglobulin (β_2-MG, mg/l) jedan je od indikatora staničnog imunosnog odgovora za koji se smatra da je biljeg aktivne parodontalne bolesti (10).

Smatra se da rana detekcija prvih kliničkih znakova parodontalne bolesti može pomoći u prevenciji i liječenju te kronične bolesti. Ovo pokusno istraživanje pokušaj je da se razjasni moguća upotreba i detekcija nekih upalnih medijatora u slini kao čimbenika rizika te indikatora rizika kad je riječ o parodontitisu.

U našem istraživanju glavni je zadatak bio procijeniti ima li upalnih medijatora u cjelokupnoj slini pacijenata s ranijim dokazima parodontitisa. Mjerili smo koncentraciju sljedećih kemokina: ALP-a, β_2-MG-a, CRP-a, LF-a, MPO-a, IL-1 te GRO-α. Dodatno smo pokušali povezati njihovu pojavu s mogućim promjenama vrijednosti kliničkih indeksa parodontalne bolesti mjerenih neposredno prije parodontalne terapije te nakon osmotjednog cijeljenja kako bismo ustanovili jesu li promjene u koncentracijama povezane s gingivalnom upalom. Takva je upala glavni uvjet za bilo koju procjenu molekularnih čimbenika/indikatora rizika za sklonost prema parodontalnoj bolesti.

Materijal i metode

Odabir ispitanika i parodontalna mjerenja

Svi ispitanici potpisali su pristanak prema Drugoj helsinškoj deklaraciji. Ispitna skupina sastojala se od 16 sudionika -11 žena i 5 muškaraca - u dobi od 23 do 53 godine (srednja vrijednost 36,6±9 godina) s najmanje 26 zuba u objema čeljustima i uznapredovalom parodontalnom bolesti. Odabrani su bili slučajno među pacijentima upućenima na Zavod za parodontologiju i Zavod za oralnu medicinu Stomatološkog fakulteta Sveučilišta u Zagrebu radi specijalističkog mišljenja i liječenja. Ni je-

about the function of GRO, although its role in chemotaxis of neutrophils and its inflammatory mediation characteristics has been established. In a human gingival fibroblast culture it has been found to be induced by IL-1β (8), and it is produced by macrophages, monocytes, neutrophils, fibroblasts, epithelial and endothelial cells, chondrocytes and osteocytes. C-reactive protein (CRP, mg/l) has been implicated as a possible mediator of the association between periodontitis and several systemic diseases, and is present in periodontally active tissue lesions (9). β_2-microglobulin (β_2-MG, mg/l) is one of the cellular immune-response indicators that is considered to serve as possible marker of active periodontal disease (10).

It is considered that early detection of first recognizable clinical signs of periodontal disease may help in the prevention and treatment of this chronic disease. This pilot study is an attempt to elucidate the possible use and detection of some salivary inflammatory mediators as risk factors or risk indicators for periodontitis.

In this study, our main aim was to assess whether the following inflammatory mediators are present in the whole saliva of the patients with previous evidence of periodontitis. We measured the concentrations of mediators in patients with periodontal disease at baseline and eight weeks after the completion of initial periodontal treatment: ALP,β_2-MG, CRP, LF, MPO, IL-1, and GRO-α. Additionally, we have attempted to correlate their presence with possible changes in values of clinical indices of periodontal disease measured at the sites immediately before periodontal treatment and after 8 weeks of healing to see whether the concentrations of the mediators were associated with gingival inflammation. Such inflammation represents an essential pre-requisite for any evaluation of molecular risk factors/risk indicators for susceptibility to periodontal disease.

Material and Methods

Subject selection and periodontal measurements

All subjects have signed a written consent according to the Helsinki declaration II. Study group consisted of sixteen, otherwise healthy, subjects (11 females and 5 males) aged 23 to 53 years (median 36 years) with at least 26 teeth in both jaws and advanced periodontal disease, randomly selected from the patients referred to the Department of Periodontology and Department of Oral Medicine, School of Dental Medicine in Zagreb, Croatia for specialist opinion and treatment. None of the subjects had

dan ispitanik nije imao nikakvu sistemsku bolest ili stanje te nije uzimao lijekove koji bi mogli utjecati na parodontalno liječenje šest mjeseci prije početka istraživanja. Svi su bili pušači, s medijanom od 11 godina pušačkoga staža (raspon od 6 do 18 godina). Ni jedan pacijent iz Zavoda za oralnu medicinu nije bolovao od oralnih mukozalnih bolesti.

Dijagnostički kriteriji za parodontalnu bolest određeni su bili prema protokolu Stomatološkog fakulteta u Zagrebu (9). Nakon pristanka svi su pacijenti dobili podrobne upute o oralnoj higijeni te je provedena subgingivalna parodontalna terapija – ostrugani su i polirani subgingivalni dijelovi zuba, uklonjen karijes te sanirani stari ili neodgovarajući ispuni. Nakon dva tjedna intenzivne higijene pacijenti su pregledani kako bi se ustanovila klinička dubina sondiranja (CPD) i vrijednosti indeksa Approximal Plaque (10) te Papilla-Bleeding (11) na svim zubima. Ta su mjerenja zabilježena kao početna (BL). Pacijenti su zatim prošli sveobuhvatnu parodontalnu terapiju, a sastojala se od struganja i poliranja korijenova supragingivalnih i subgingivalnih dijelova zuba, uklanjanja karijesa te zamjene starih i loših ispuna. Osam tjedana nakon završetka terapije svi su sudionici bili ponovno pozvani i pregledani, a izmjereni su im opet i klinički parametri. Ta su mjerenja zabilježena kao osam tjedana (8W).

Prikupljanje i skladištenje sline

Količina sline prikupljena je tijekom pet konsekutivnih mjerenja nakon završetka programa oralne higijene (BL) te osam tjedana nakon završetka parodontalne terapije. Ispitanici su sjedili u stomatološkom stolcu uspravno i pet minuta izbacivali slinu u kalibrirane epruvete (0,1 ml), ponavljajući postupak četiri puta (12). Slina je do analiziranja bila skladištena na –70˚C.

Laboratorijska analiza uzoraka sline

Za analizu MPO-a primijenjena je bila ELISA, prema prije detaljno opisanom postupku (13). Isti je postupak bio i kod identifikacije i mjerenja LF-a (14) i ALP-a (15). GRO-α identificiran je i analiziran indirektnom imunološkom pretragom pomoću monoklonalnih antitijela (16). IL-1 identificiran je testom IL-1 ELISA (Cistron Biotechnology, Pine Brook, NJ, SAD - dobavljač Medika d.d., Zagreb, Hrvatska). Postupak je potanko opisan u radu Wilsona i suradnika (17). Analiza β_2-MG-a obavljena je postupkom koji su detaljno opisali Cooper i njegovi kolege (18) - radioimunološkim mjerenjem dvostrukih koncentracija. CRP je mjeren radijalnom imunodifuzijom pomoću antisera i standarda (Behany systemic disease or condition, nor did they take any medications that might have influenced the periodontal treatment 6 months before baseline. All subjects were smokers, with median of 11 years of smoking (range 6 to 18 years). None of the patients referred to the Department of Oral Medicine have had any oral mucosal diseases.

Criteria used for the diagnosis of periodontal disease were according to established protocol of the School of Dental Medicine and American Academy of Periodontology (9). After acceptance, all patients received thorough oral hygiene instructions. After two weeks of intensive hygiene program, all patients were examined in order to establish clinical probing depth (CPD), Approximal Plaque Index (API) (10) and Papilla-Bleeding Index (PBI) (11) on all present teeth. These measurements were recorded as baseline (BL). The patients were subsequently submitted to comprehensive periodontal therapy that consisted of scaling and root planing of the supragingival and subgingival parts of the teeth, caries removal and replacement of faulty or old restorations. Eight weeks after completion of the therapy, all patients were recalled, and the periodontal examination was repeated. These measurements were recorded as eight weeks (8W).

Saliva collection and storage

The amount of saliva was collected during five consecutive measurements after completion of oral hygiene program (BL) and eight weeks after the completion of periodontal therapy. The subjects were seated upright in dental chairs, expectorating saliva in calibrated tubes (9 ml volume) during 5 minutes, and repeating the procedure four times (12). Whole saliva samples were stored at –70˚C until analysis.

Laboratory analysis of saliva samples

ELISA was performed for assay of MPO by procedures described elsewhere in detail (13). Same procedures were used for LF (14) and ALP (15) identification and measurements. GRO-α was identified and analyzed by indirect immunodots using monoclonal antibodies (16). IL-1 assay was performed using the IL-1 ELISA test (Cistron Biotechnology, Pine Brook, NJ, USA, supplied by Medika d.d., Zagreb, Croatia). The procedure was described in detail by Wilton et al. (17). β_2-MG assays were performed using the procedure described in detail by Cooper et al. (18), by radioimmunoassay measurement of duplicate concentrations. CRP was measured by radial immunodiffusion using antisera

ringwerke, Marburg/Lahn, Njemačka - dobavljač Medika d.d., Zagreb, Hrvatska).

Statistička analiza

Laboratorijski rezultati imali su normalnu distribuciju te su predstavljeni pomoću srednjih vrijednosti i standardne devijacije, a podaci su uspoređeni korelacijskom analizom i parnim parametrijskim testovima. Velikima su smatrane samo P vrijednosti niže od 0,05.

Rezultati

Incijalne vrijednosti API i PBI bile su znatno različite od vrijednosti 8W (P <0,05, tablica 1). Slike od 1 do 4 pokazuju srednje vrijednosti svih

and standards (Behringwerke, Marburg/Lahn, Germany, supplied by Medika d.d., Zagreb, Croatia).

Statistical analysis

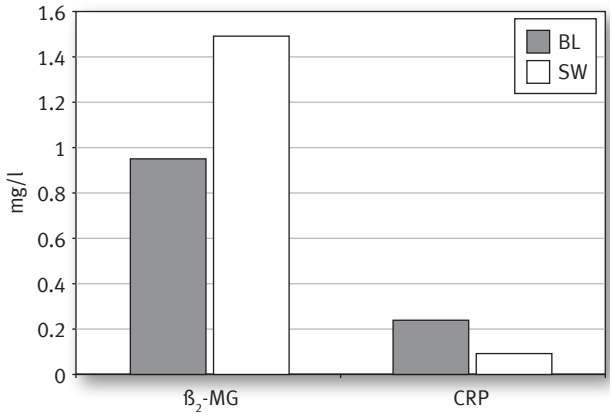
Laboratory results were distributed according to normal distribution, presented with mean and standard deviation and data compared using paired parametric statistics and correlation analysis. Only P values lower than 0.05 were considered significant.

Results

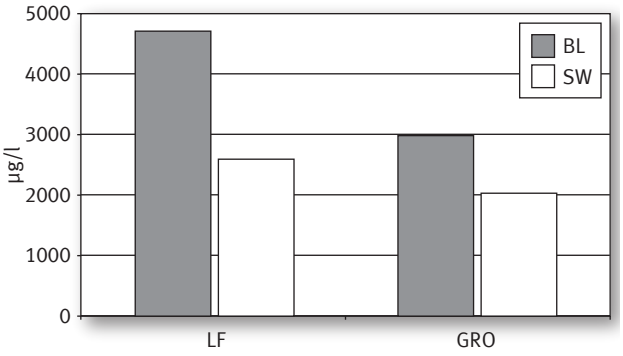
Initial API and PBI values were significantly different compared to values at 8W (P <0.05, Table 1). Figures 1 to 4 show mean values of all recorded in-

Tablica 1. Podaci kliničkih nalaza (srednja vrijednost ± standardna devijacija) (*statistički znatna razlika dvaju mjerenja; P < 0.05).
Table 1 Clinical findings data (mean ± standard deviation) (*significant difference between baseline and after eight weeks measurement; P < 0.05).

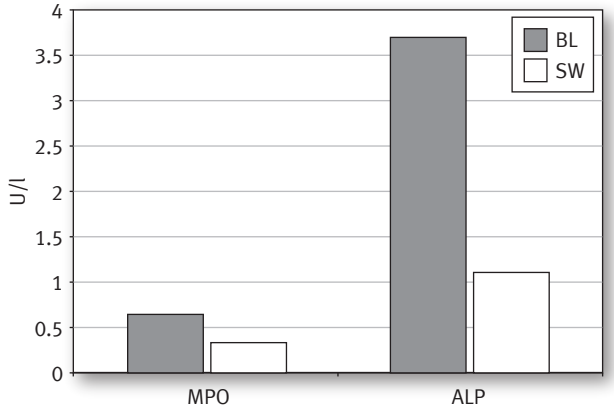
CPD (mm)		API (%)		PBI	
BL	8W	BL	8W	BL	8W
6.4±2.3	3.9±1.9*	61.5±11	31.2±11.5*	51.9±16	27.8±13.3*



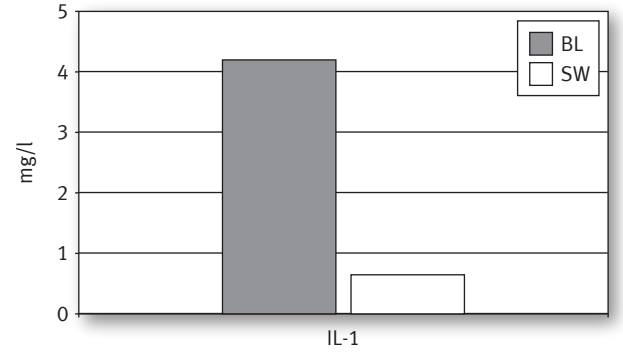
Slika 1. Srednje vrijednosti β_2-MG i CRP.
Figure 1 Mean measured values of β_2-MG and CRP.



Slika 2. Srednje vrijednosti LF i GRO.
Figure 2 Mean measured values of LF and GRO.



Slika 3. Srednje vrijednosti MPO i ALP.
Figure 3 Mean measured values of MPO and ALP.



Slika 4. Srednje vrijednosti IL-1.
Figure 4 Mean measured values of IL-1.

bilježenih upalnih medijatora u slini. Statistički velika razlika uočena je kod vrijednosti LF-a (P<0,01), MPO-a (P<0,05), IL-1 (P<0,01), ALP-a (P<0,05) i CRP-a (P<0,05) kod 8W u usporedbi s BL-om (nije opažena nikakva druga razlika; Tablica 2. srednje vrijednosti sa standardnim devijacijama za sve parametre). Tablica 3. prikazuje odnos između svih kliničkih mjerenja te koncentracije svakog upalnog medijatora na 8W. Velika i važna korelacija vrijednosti pronađena je između CPD-a i API-a, API-a i PBI-a, β_2-MG-a i PBI-a, LF-a i PBI-a, IL-a i CPD-a, ALP-a i API-a te CRP-a i CPD-a, API-a i PBI-a.

flammatory mediators in whole saliva. There was statistically significant difference between the concentrations of LF ($P < 0.01$), MPO ($P < 0.05$), IL-1 ($P < 0.01$), ALP ($P < 0.05$) and CRP ($P < 0.05$) at 8W when compared to BL (no other difference was observed; Table 2 – mean values with standard deviations of all parameters). Table 3 presents correlation between clinical measurements and concentrations of each inflammatory mediator at 8W. Important (and significant) correlation values were found between CPD and API, API and PBI, β_2-MG and PBI, LF and PBI, IL-1 and CPD, ALP and API, and CRP and CPD, API and PBI.

Tablica 2. Srednja vrijednost i standardna devijacija medijatora koncentracije (BL i 8W)
Table 2 Mean values (Mean) and standard deviations (SD) of mediator concentrations, at BL and 8W.

	Mean BL	SD BL	Mean 8W	SD 8W
β_2-MG (mg/l)	0.95	0.31	1.49	0.18
CRP (mg/l)	0.23	0.08	0.09[a]	0.01
LF (μg/l)	4774	543	2613[b]	445
GRO (ng/ml)	2967	382	2027	742
MPO (U/ml)	0.64	0.02	0.31[a]	0.05
ALP (U/l)	3.69	0.17	1.11[a]	0.38
IL-1 (μg/l)	4.15	0.02	0.67[b]	0.19

Statistički znatna razlika na razini 0.05 ili 0.01 • Statistically significant difference at 0.05[a] or 0.01[b] value.

Tablica 3. Koeficijenti korelacije s razinama znatnosti P između laboratorijskih parametara.
Table 3 Pearson correlation coefficients r with P values between laboratory parameters.

		CPD	API	PBI
API	r P	0.43 0.04	–	–
PBI	r P	0.28 0.07	0.79 0.04	–
β_2-MG	r P	0.0 0.0	0.04 0.01	-0.74 0.1
LF	r P	0.2 0.09	-0.37 0.1	0.64 0.02
MPO	r P	-0.27 0.01	0.08 0.0	-0.29 0.05
IL-1	r P	0.54 0.01	0.28 0.08	0.28 0.53
GRO	r P	-0.22 0.38	0.22 0.02	0.03 0.00
ALP	r P	0.03 0.18	0.67 0.05	-0.09 0.34
CRP	r P	0.84 0.07	0.88 0.03	0.86 0.38

Rasprava

Ovaj rad je pokusni te iz više razloga određuje smjer daljnjim istraživanjima u ovom području. Kronični gingivitis i parodontitis uobičajene su upalne bolesti, a karakterizira ih dinamička uloga neutrofila, T-limfocita i - ovisno o stanju - plazmocita. Iako je u tim bolestima općeprihvaćena uloga leukocitnih kemotaktičnih čimbenika, njihov su identitet i izvor i dalje nepoznati (19).

U kronično inflamiranoj gingivi monociti i makrofagi proizvode IL-1. U novijim istraživanjima potvrđeno je da fibroblasti proizvode i luče niz proupalnih i imunoregulatornih citokina, ponajprije IL-1, IL-6 i TNF-α. Fibroblasti u upaljenom području, zajedno s makrofagima i PMN neutrofilima, mogu tijekom parodontalne bolesti pridonijeti destrukciji vezivnog tkiva. Oni se nalaze u svim reparatornim procesima, a njihov se broj smanjuje kako napreduje cijeljenje. Longitudinalna istraživanja mogla bi pridonijeti važnosti prisutnosti ili odsutnosti IL-1 u ukupnoj slini pacijenata s destruktivnom parodontalnom bolesti. IL-1 u slini pacijenata s dokumentiranom parodontalnom bolesti vjerojatno potječe iz lokalno nakupljenih fibroblasta i keratinocita, ili stanica iz vaskulature. Iako se nalazi i u plazmi (22), pretpostavljamo da se veći dio sintetizira lokalno.

Pretpostavlja se da koncentracije medijatora u ukupnoj slini mogu u trenutku kontrole biti pripisane potpunom cijeljenju.

Povećane vrijednosti MPO-a mogu se pripisati degranulaciji neutrofila uzrokovanoj specifičnim bakterijskim toksinima koji se mogu naći na zahvaćenim mjestima i u slini. Takvi mikroorganizmi, kao *Aggregatibacter actinomycetemcomitans*, mogu uzrokovati smrt neutrofila sekrecijom toksina (leukotoksina, epiteliotoksina, fibrinolizina) što dovodi do lize stanica (23). Uzorci ukupne sline uglavnom imaju niske vrijednosti MPO-a zbog razrjeđenja sulkularne tekućine (23). Naši su rezultati suprotni, iako nije jasno što je uzrokovalo tako visoke vrijednosti MPO-a u uzorcima ukupne sline. Povezanost MPO-a i parodontitisa dobro je ustanovljena, a visoke koncentracije MPO-a u slini upućuju na to da je povezan i s jačinom te vrstom destrukcije parodontalnih tkiva (23,24).

Wolff i suradnici (25) opisali su povišene koncentracije salivarnog IL-1α kod ljudi s gingivitisom ili parodontitisom u odnosu prema zdravoj kontrolnoj skupini, što je u skladu s našim rezultatima. Nema konzistentnih istraživanja kemokina u parodontitisu, te se dobivene vrijednosti ne mogu uspoređivati s prijašnjim studijama, pa to umanjuje

Discussion

This report should serve as a pilot paving the way for future research in this field, for many reasons. Chronic gingivitis and periodontitis are characterized by dynamic involvement of neutrophils, T-lymphocytes, and, depending on the status, plasmocytes. Although the existence and the possible role of leukocytic chemotactic factors in these diseases have been widely accepted, their identity and the source remain unknown (19).

In chronically inflamed gingiva monocytes/macrophages produce IL-1. Recent research has shown that fibroblasts produce and secrete a series of proinflammatory and immunoregulatory cytokines, such as IL-1, IL-6 and TNF-α. Fibroblasts present at the inflammatory site, as well as activated macrophages and PMN-neutrophils, can contribute to connective tissue destruction in periodontal disease. Fibroblasts are present in all reparatory processes, and their number decreases as the healing process continues. Longitudinal data can contribute to the meaning of presence or absence of IL-1 in whole saliva of patients with previous destructive periodontal disease. IL-1 in saliva of patients with documented periodontal disease probably originates from locally aggregated fibroblasts and keratinocytes, or from cells emigrating from the vasculature. Although IL-1 can be found in plasma as well (22), we are presuming that most of the salivary IL-1 is synthesized locally.

It is plausible that concentrations of mediators in whole saliva can be attributed to the complete healing at the time of control.

Increased values of MPO can be attributed to neutrophil degranulation that is caused by specific bacterial toxins found in affected sites, and saliva. Such microorganisms, like *Aggregatibacter actinomycetemcomitans*, can cause cytopathic changes in neutrophils by secretion of its toxins (leukotoxin, epitheliotoxin, fibrinolysin), leading to cell lysis (23). Whole saliva samples usually have lower MPO activity, due to the fact of dilution of crevicular fluid in saliva (23). We are reporting different findings, although it is not clear what caused such high concentrations of MPO in whole saliva samples. The relationship between MPO and periodontitis is firmly established, and reported high MPO concentrations in whole saliva suggests that MPO may be related to the pattern and severity of the periodontal breakdown as well (23,24).

Wolff et al. (25) have reported higher concentrations of salivary IL-1α in persons with gingivitis

vrijednost istraživanja. U ranijem istraživanju bilo je istaknuto da visoka koncentracija β_2-MG može upozoravati na patogenezu parodontalne bolesti (26), a Mogi i suradnici (10) ponovili su da su visoke koncentracije prisutne u upalnim i eksudativnim reakcijama u parodontalnom džepu. Smanjenje bi moglo biti rezultat sveopćih bioloških odgovora tijekom cijeljenja nakon parodontalne terapije, kao što je to bilo u našem istraživanju.

Kad je riječ o vrijednostima CRP-a, podaci zbunjuju. Nedavno objavljeno istraživanje japanskog znanstvenika Yamazakija i njegovih kolega (27) pokazalo je da nema većeg utjecaja parodontalne bolesti na serumske vrijednosti CRP-a. No, lokalno te vrijednosti mogu porasti tijekom akutne upale u parodontalnim tkivima (28). Vrijednosti u ukupnoj slini samo odražavaju vrijednosti iz sulkularne tekućine, a naši su rezultati u skladu s dosadašnjima.

Komine i suradnici (29) opisali su inflamatorne peptide LF-a u parotidnoj slini pacijenata s parodontitisom. Naši se rezultati slažu s njihovima i pokazuju povećane vrijednosti LF-a u aktivnoj parodontalnoj bolesti. Čini se da je koncentracija LF-a povezana s težinom kliničkih simptoma. Mjerenje upalnog LF-a moglo bi biti korisno u dijagnostici parodontitisa (29).

Dobro je poznato da neke sastavnice sline potječu iz gingivalne sulkularne tekućine. Procjena rizika može pomoći u prevenciji parodontalne bolesti, no velike su teškoće koje se mogu pojaviti tijekom postupaka ranog otkrivanja. Indikatori rizika su čimbenici koji ne moraju biti etiološki, no sigurno su povezani s progresijom bolesti (22). Kada bismo pretpostavili da različiti medijatori upale imaju općenitu i dobro definiranu zadaću, to bi bilo prejednostavno ili potpuno netočno. Prikupljanje podrobnih informacija o njima i dalje predstavlja izazov, a ograničeno je njihovom malom ekspresijom toliko uobičajenom za citokine. Na kraju, čini se da - kao i ostale bjelančevine u ljudskom tijelu - upalni medijatori imaju različite funkcije u različitim tkivima i stanjima.

Nemoguće je ispitivati samo neke upalne medijatore u parodontalnoj bolesti. Uključe li se i drugi upalni čimbenici, u prvom redu prostaglandini, jasno je da naši podaci ne čine cijeli mehanizam koji završava razaranjem parodontalnih tkiva. No, kompletna mreža citokina uključena u patogenezu parodontalne destrukcije, osigurava dovoljnu stimulaciju za istraživanja, a to je samo jedan pokušaj da se pridonese znanstvenim spoznajama u tom području.

or periodontitis when compared to healthy controls, which is in accordance with our results. There is no consistent research on chemokines in periodontitis so far, and the values that are reported cannot be compared to earlier studies, thus having rather low importance. A previous investigation suggested that high concentration of β_2-MG could reflect the pathogenesis of periodontal disease (26), and Mogi et al. (10) reiterated that high concentrations are present in inflammatory and exudative reactions in periodontal pockets. Its decrease might also reflect the comprehensive biological responses during healing after periodontal therapy, as was shown in our study.

Regarding CRP values, the data is confusing. A recent Japanese study (27) showed that there is no significant influence of periodontal disease on serum levels of CRP. However, locally the values of CRP can be increased during acute inflammation in periodontal tissues (28). Whole saliva values reflect GCF values, and our results are in accordance with these.

Komine et al. (29) reported on cleaved inflammatory LF peptides in parotid saliva of periodontitis patients. Our results are in accordance with theirs, showing increased presence of LF in active periodontal disease. It seems that the LF concentration is correlated to the severity of clinical symptoms. Measurement of inflammatory LF might be useful for diagnosis of periodontitis (29).

It is well known that some salivary constituents actually derive from gingival crevicular fluid. Risk assessment may help in the prevention of periodontal disease, but difficulties that may arise in procedures of early detection are great. Risk indicators are factors that are not necessarily present as etiologic factors, but are certainly associated with the disease progression (22). To consider that different inflammatory mediators have a general and well-defined role would be too simple, or completely wrong. Gathering of complete information on them remains a challenge, limited by their low levels of expression that is usual for most of the cytokines. Finally, it seems that, like most other proteins in the human body, inflammatory mediators have different functions in different tissues and conditions.

It is impossible to investigate just some of the inflammatory mediators in the periodontal disease. When other pro-inflammatory factors, notably prostaglandins, are also taken into account, it is clear that our data do not show all the molecules in the pathway that leads to periodontal breakdown. However, whole cytokine network in the pathogenesis of

Zaključak

Ovo istraživanje otkrilo je da koncentracije različitih proupalnih citokina variraju s obzirom na parodontalno cijeljenje. Koncetracije CRP-a, LF-a, GRO-a, MPO-a, IL-1 i ALP-a smanjile su se s parodontalnim cijeljenjem, a povećana je bila koncentracija β_2-MG-a. Iako je potrebno daljnje istraživanje, može se zaključiti da koncentracije citokina mogu biti pouzdani indikatori rizika za aktivnu parodontalnu bolest.

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periodontal destruction provides much stimulation to the research, and this is an attempt to add to the scientific knowledge in this field.

Conclusions

Our study reveals that concentrations of different pro-inflammatory cytokines vary regarding to periodontal healing. Concentrations of CRP, LF, GRO, MPO, IL-1 and ALP decreased with periodontal healing, while the concentration of β_2-MG was increased. Although further research is needed, it may be concluded that cytokine concentrations can prove to be a reliable risk indicator for active periodontal disease.

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Abstract

Purpose: Little is known on levels of inflammatory mediators in saliva in periodontitis-affected individuals. **Material and Methods:** In order to establish the pattern of their concentrations in different stages of periodontal healing process, we have measured the levels of alkaline phosphatase (ALP), lactoferrin (LF), myeloperoxidase (MPO), interleukin-1 (IL-1), and GRO-α in patients with previous periodontal disease. Whole saliva samples were collected immediately following, as well as eight weeks after periodontal treatment in a group of 16 subjects with clinical signs and symptoms of periodontitis. **Results:** Statistical analysis revealed significant differences of clinical periodontal parameters and in levels of mediators between two measurements. Concentrations of CRP, LF, GRO, MPO, IL-1 and ALP decreased with periodontal healing, while the concentration of β_2-MG was increased. **Conclusion:** It may be concluded that cytokine concentrations are possible risk indicators for active periodontal disease.

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Key words
Periodontitis; Saliva; Alkaline Phosphatase; Lactoferrin; Peroxidase; Interleukin-1; Enzyme-Linked Immunosorbent Assay; Citokines

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