Primljen/ Recived on : 19.03.2015.
Revidiran/ Revised on : 26.03.2015.
Prihvaćen/ Accepted on : 08.04.2015.

INFORMATIVNI RAD
INFORMATIVE ARTICLE
doi:10.5937/asn1571473S

MEDIJATORI OTKRIVENI U GINGIVALNOJ TEČNOSTI I PLJUVAČKI KAO BIOMARKERI PARODONTOPATIJE

MEDIATORS DETECTED IN GINGIVAL FLUID AND SALIVA AS BIOMARKERS OF PERIODONTAL DISEASE

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Sažetak

Paradontopatija je skup inflamatornih oboljenja koje karakteriše gubitak vezivno tkivnog pripoja i kosti oko zuba, što dovodi do pomeranja epitela i formiranja dubokih parodontalnih džepova. Ako se ne leči, može dovesti do slabljenja a samim tim i do gubitka zuba. Rana dijagnoza i lečenje progresivne parodontopatije su važni zbog nepovratne prirode ove bolesti. Dugoročni cilj je lečenje i sprečavanje parodontopatije. Klinička merenja korišćena u dijagnostici parodontalnih oboljenja su često ograničenih mogućnosti i predstavljaju indikatore o prethodnom oboljenju, a ne indikatore o sadašnjoj aktivnosti bolesti. Razvoj testa za mnoge medijatore koji su u vezi sa parodontopatijom može poslužiti kao koristan metod za identifikaciju i predviđanje buduće progresije. Biohemijski posrednici u oralnim tečnostima, kao što su pljuvačka i gingivalna tečnost, veoma su korisni zbog neinvazivne i jednostavne prirode njihove primene. Oni pomažu u određivanju nivoa upalnih medijatora, jer su dobri pokazatelji inflamatorne aktivnosti. Ovaj pregled analizira potencijalni niz pljuvačnih i gingivalno-fluidnih biomarkera, na osnovu dijagnoze i prognoze aktivne parodontopatije.

Ključne riječi: parodontopatija, biomarkeri, gingivalna tečnost, pljuvačka

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Abstract

Periodontitis is a set of inflammatory diseases characterized by the loss of connective tissue attachment and supporting bone around the teeth, which results in the apical migration of the junctional epithelium and the formation of deepened periodontal pockets. If left untreated, it can lead to the loosening and subsequent loss of teeth. Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease. Thus the long-term aim is the treatment and prevention of periodontal disease. Clinical measurements used in the diagnosis of periodontal diseases are often of limited usefulness in that they are indications of previous periodontal disease rather than present disease activity. The development of a test for most mediators associated with periodontal disease may serve as a useful method for identifying and predicting future progression. Biochemical mediators in oral fluids such as saliva and gingival crevicular fluid are highly beneficial, due to the non-invasive and simple nature of their collection. They help in the determination of inflammatory mediator levels, as they are good indicators of inflammatory activity. This review analyzed the potential array of salivary and gingival crevicular fluid biomarkers, based on the diagnosis and prognosis of active periodontal disease.

Key words: periodontal disease, biomarkers, gingival crevicular fluid, saliva

Uvod

Parodontopatije predstavljaju raznovrsne heterogene inflamatorne bolesti koje utiču na pripoj vezivnog tkiva i potpornu kost oko zuba[1]. Mikrobiološka priroda ove bolesti je odavno ispitana, dok nedavne studije ukazuju na faktore koji se dovode u vezu sa odgovorom domaćina na patogenu infekciju [2,3]. To je presudni faktor za razumevanje progresije bolesti. Faktori bakterijske virulencije utiču na oslobađanje bioloških medijatora iz ćelija tkiva domaćina koje su i dovele do uništenja tkiva. Bakterijski faktori virulencije generišu oslobađanje bioloških medijatora iz ćelija tkiva domaćina, koji su u stvari doveli do uništenja tkiva[4]. To su: proteinaze, citokini i prostaglandini. Takođe, mnogi enzimi oslobođeni preko periodontalnih mikroorganizama uništavaju tkiva [5]. Lokalno prisustvo bakterija u tečnosti gingive i bliski kontakt bakterijskih lipopolisaharida sa ćelijama domaćina povećava priliv ćelija (neutrofila i monocita-makrofaga i drugih iz periferne krvi). Nakon toga, T ćelije i B ćelije proizvode inflamatorne citokine kao što su IL-1, TNF-alfa i prostaglandin E-2. Važnu ulogu u destrukciji parodontalnog tkiva imaju IL-1 i TNF-alfa, a PGE-2 je delimično odgovoran za gubitak koštane mase parodonta koji se dovodi u vezu sa bolešću [6].

Rana dijagnoza i lečenje progresivne parodontopatije su veoma važni zbog recidiva bolesti. Svrha parodontalnih dijagnostičkih procedura je da pruži korisne informacije za lekara u vezi sa vrstom bolesti, njenom lokacijom i težinom. Ova otkrića koriste se kao osnova za planiranje parodontološkog tretmana i obezbeđivanje bitnih podataka tokom parodontološkog monitoringa, kao i za praćenje faza lečenja bolesti. Tradicionalni klinički pokazatelji (sondiranje parodontalnih džepova, detekcija krvarenja pri sondiranju, klinički gubitak pripoja, indeks plaka i radiografija) koji se koriste više od 50 godina i dalje funkcionišu kao osnovni model parodontološke dijagnoze[7]. Danas je dijagnoza parodontopatije sve više ograničena, jer se uglavnom zasniva na odbrambenim mehanizmima domaćina koji su predhodili nastanku paradontopatije, a ne na trenutnoj aktivnosti bolesti. Prednosti oralnih i periodontalnih dijagnostičkim dostignuća su otkrivanje novih metoda gde se rizik od bolesti može prepoznati i kvantifikovati kroz objektivne parametare poput biomarkera. Biomarkeri se mogu definisati

Introduction

Periodontal diseases represents heterogeneous, inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth[1]. The microbiological nature of this disease was investigated a long time ago, while the latest studies show factors associated with the host response to the pathogenic infection [2,3]. It is a critical factor to disease progression that in fact would be the key to understanding the processes of its progression. Bacterial virulence factors generate the release of biological mediators from host tissue cells, which in fact lead to tissue destruction [4]. These are: proteinases, cytokines and prostaglandins. Also, many enzymes produced by periodontal microorganisms lead to tissue destruction[5]. The local presence of bacteria near gingival fluid and intimate contact with bacterial lipopolysaccharide with the host cells enhances the influx of cells (neutrophils and monocyte-macrophages and others from the peripheral blood). Subsequently, T cells and B cells produce inflammatory cytokines such as IL-1, TNF-alpha and prostaglandin E-2. IL-1 and TNF-alpha have an important role in periodontal tissue destruction, and PGE-2 appears to be partially responsible for periodontal bone loss associated with the disease [6].

The early diagnosis and treatment of progressive periodontal disease is very important due to the recurrent nature of the disease. The purpose of periodontal diagnostic procedures is to provide useful information for the clinician regarding the type of disease, location, and its severity. These discoveries are used as a base for planning the treatment and provision of essential dates during periodontal maintenance and monitoring of the phases of treatment of the disease. Traditional clinical indicators (probing of periodontal pocket depths, bleeding on probing, clinical attachment loss, plaque index and radiography), which have been used for more than 50 years, continue to function as a basic model for periodontal diagnosis[7]. Today, periodontal diagnosis is more and

kao supstancije koje su objektivno merljive. Vrednuju se kao indikatori normalnih bioloških procesa, patoloških procesa i farmakoloških odgovora na terapijsku intervenciju. Biomarkeri, samostalno proizvedeni, kod zdravih pojedinaca ili pojedinaca sa specifičnim sistemskim oboljenjima, su znak-molekuli, koji se mogu iskoristiti u praćenju zdravstvenog stanja, statusa bolesti, odgovora lečenja i terapijskih rezultata. Biomarkeri preuzmaju ulogu u rutinskoj praksi dijagnostikovanja, praćenju terapijskih rezultata i otkrićima lekova[8,9,10]. Značajni pomaci su postignuti u poslednjih 10 godina analiziranjem pljuvačke, gingivalne tečnosti i mukoznog transudanta, koji predstavljaju biološke uzorke za otkrivanje oralnih i sistemskih bolesti. Lako prikupljeni, sadrže lokalne i sistemski oslobođene biomarkere, koji mogu ponuditi osnovu dijagnostičkih specifičnih testova parodontopatije. Ovaj članak naglašava napredak u korišćenju medijatora koji su identifikovani u gingivalnom fluidu i pljuvački kao biomarkerima parodontopatije.

Potencijalni biomarkeri parodontopatije u tečnosti gingive

Gingivalna tečnost je eksudat gingivalne mikrocirkulacije koja prolazi kroz zdravo i inflamirano parodontalno tkivo. Sastoji se od seruma i lokalno generisanih materijala, kao što su proizvodi tkivnog razgrađivanja, inflamatorni medijatori i antitela usmerena ka bakterijama zubnog plaka. Sastojci domaćina uključuju molekule iz krvi i tkiva, periodontalni epitel, minerale i mineralno vezivno tkivo, kao i inflamatorne i imune ćelije koje infiltriraju parodont. Celularne komponente u tečnosti gingive čine 70-80% granulocita, 10-20% monocita/makrofaga, 5% mastocita i 5% T-limfocita. Prikupljanje i analiza uzoraka gingivalne tečnosti služi kao neinvazivno kvantitativni biohemijski pokazatelj za procenu lokalnog ćelijskog metabolizma koji odražava parodontalno zdravstveno stanje neke osobe [11,12,13]. Gingivalna tečnost se lako prikupiti periofilter trakama i mikrokivetama iz gingivalnog sulkusa.

more limited, because they are mainly the indicators of previous activities for preserving from the disease, rather than its present activity. The advantages in oral and periodontal diagnostic achievements are discovering new methods where the risk of the disease can be identified and quantified through objective parameters just like biomarkers. Biomarkers can be defined as substances that are objectively measurable and valued as indicators of normal biological processes, pathological processes and pharmacological responses to therapeutic interventions. Biomarkers, if independently produced, in healthy individuals or individuals affected by a specific systemic diseases, are sign-molecules that can be exploited in monitoring the health status, the status of the disease, the response of the treatment and the results. The challenge for biomarkers is to assume a role in routine practice diagnosis, monitoring and therapeutic results and the discovery of medicines[8,9,10]. Significant advances have been achieved within the past 10 years using saliva, gingival crevicular fluid (GCF), and mucosal transudate as biological samples for the detection of oral and systemic illnesses. Easily collected and containing local and systemically derived biomarkers of periodontal disease, oral fluids may offer the basis for a patient-specific diagnostic test for periodontal disease. This article highlights advances in the use of the identification of mediators that are released into GCF and saliva as biomarkers of periodontal disease.

Potential biomarkers for periodontal disease in gingival fluid

Gingival fluid is an exudate of gingival microcirculation which passes through healthy and inflamed periodontal tissue. It is composed of serum and locally generated materials, such as tissue break-down products, inflammatory mediators and antibodies directed against dental plaque bacteria. Constituents of the host include molecules from the blood and contributions from the cells and the periodontal tissues, epithelium, mineral and mineral connective tissue, as well as inflammatory and immune cells that infiltrate into the periodontal tissue.

Gingivalna tečnost je detaljno ispitivana u vezi sa oslobađanjem faktora domaćina. U skladu sa studijom Armitage[14], preko 65 sastojaka iz gingivnog fluida procenjuju se kao potencijalni dijagnostički markeri u progresiji parodontopatije. Ovi markeri se mogu podeliti u tri grupe: domaćin izvedeni enzimi i njihovi inhibitori, medijatori inflamacije i domaćin-odgovor modifikatori i produkti tkivnog raspadanja.

Neutrofilni medijatori, identifikovani u tečnosti gingive, uključuju: leukotrien B4 (LTB4), PAF, tromboksan B2, elastaze i kolagenaze [15, 16].

Monociti (makrofage) luče inflamatorne medijatore poput PGE2, IL-1, IL-6, IL-8 i TNF. Mnogi istraživači su pokazali korelaciju između kolagenolitične aktivnosti i ozbiljnosti parodontopatije. Villela i saradnici su takođe primetili vezu između kolagenolitične aktivnosti i aktivnosti oboljenja[17]. Kao rezultat ovih ranih posmatranja nije se razmišljalo o mogućoj upotrebi kolagenaze kao biohemijskog markera progresije bolesti.

Prostaglandini su metaboliti arahidonske kiseline koji se sastoje od 10 klasa, od centracije PGE2 kod pacijenata sa gingivitisom u poređenju sa pacijentima periodontalne bolesti. Njegova grupa je naknadno obavkojih su D, E, F, G, H i I od velike važnosti. Iz ove grupe, PGE2 je jedan od najčešće ispitivanih medijatora aktivnosti parodontalnog oboljenja [18, 19]. U patogenezu parodontopatije uključen je PGE2, prvobitno identifikovan u gingivalnoj tečnosti sredinom 1970-ih, a kasnije bio ispitivan u odnosu na parodontopatiju. Offenbacher i sar. su potvrdili da postoje razlike u gingivalnom fluidu konila retrospektivnu analizu PGE2 ispitivanjem uzdužnog odnosa koncentracija PGE2 u gingivalnom fluidu i gubitka pripaja kod odraslih pacijenata sa parodontopatijom. Rezultati su pokazali da je povišeni PGE2 bio merljiv u gingivalnom fluidu 6 meseci pre identifikacije parodontalne aktivnosti bolesti, a signifikantno smanjen posle jednog meseca nakon terapije[20]. PGE2 deluje kao potentni vazodilatator i povećava kapilarnu nepropustljivost, kapilarnu nepropustljivost, što izaziva kliničke znake

Cellular components in the gingival fluid include granulocytes (10-20%), monocytes /macrophages (10-20%), mastocytes (5%), and T-lymphocytes (5%). Collection and analysis of gingival fluid samples provide a non-invasive quantitative biochemical indicator for the evaluation of the local cellular metabolism that reflects a person's periodontal health status[11,12,13]. Gingival fluid can be easily collected with periofilter strips, absorbing point paper and microcivetes from the gingiva hole around the teeth.

GCF has been extensively investigated for the release of the host response factors. In accordance with the Armitage study[14], more than 65 gingival fluid constituents are evaluated as potential diagnostic markers in the progression of periodontal disease. These markers can be divided into three groups: host-derived enzymes and their inhibitors, inflammatory mediators and host-response modifiers and tissue breakdown products.

Neutrophil mediators identified in gingival fluid include: leukotriene B4 (LTB4), PAF, thromboxane B2, elastase and collagenase [15, 16].

Monocytes (macrophages) secrete inflammatory mediators such as PGE2, IL-1, IL-6, IL-8 and TNF. Many researchers have demonstrated a correlation between the collagenolytic activity of GCF and the severity of periodontal disease. Villela and co-workers also observed a connection between collagenolitic activity and active disease[17]. As a result of these early observations a, thought was given to the possible use of collagenases as possible biochemical markers for disease progression.

Prostaglandins are arachidonic acid metabolites composed of 10 classes, of which D, E, F, G, H, and I are of main importance. Of this group, PGE2 is one of the most extensively studied mediators of periodontal disease activity[18,19]. PGE2, involved in the pathogenesis of periodontal disease, was originally identified in the gingival fluid in the middle of 1970s and later studied in relation to periodontal disease. Offenbacher et al. confirmed that there are differences in the gingival fluid concentration of PGE2 in patients with gingivitis compared with periodontal disease patients. His group subsequently performed a

crvenila i edema. PGE2, takođe, stimuliše fibroblaste i osteoklaste da bi se povećala proizvodnja MMP. Većina kliničkih ispitivanja istražila je upotrebu COKS-2 inhibitora, kao dodatka periodontalne terapije. Ovi inhibitori poboljšavaju klinički ishod nakon parodontološke terapije u poređenju samo sa parodontalnom terapijom[21,22]. Ove studije pokazuju da prostaglandini, naročito PGE-2, mogu biti uključeni u paradontopatiji izazvan resorpcijom kosti. Na kraju, MMP utiču na remodelovanje i degradaciju periodoncijuma. Kasnije se utvrdilo da postoji korelacija između povećane koncentracije PGE2 i kliničkog gubitka pripoja kod pacijenata sa umerenom ili uznapredovalom parodontopatijom. Ipak, PGE2 se pokazao kao značajan biomarker parodontopatije.

Proinflamatorni citokini, posebno IL-1 beta, mogu da igraju važnu ulogu u etiologiji parodontopatije, tako da je težina parodontopatije povezana sa lokalnim fluidnim povećanjem IL1-beta, TNF-alfa i prostaglandina, kao što su PGE2, dok inhibicija ovih supstanci proizvodi značajno smanjenje parodontopatije. Liu i sar. su potvrdili da sa porastom gingivalnog indeksa inflamacije i povećanja dubine parodontalnog džepa postoji odgovarajuće povećanje koncentracije IL1-beta u gingivalnoj tečnosti [23,24]. Stefanovska u svojoj studiji ukazuje na značajno povećanje koncentracija IL1-alfa i IL1-beta u tečnosti gingive sa porastom stepena gingivalne inflamacije [25]. Loos i Toja [16] analiziraju potencijalne dijagnostičke markere u gingivalnoj tečnosti kod pacijenata sa parodontopatijom. Njihova istraživanja identifikuju 8 potencijalno korisnih markera uključujući: alkalne fosfataze, beta-glukuronidase, katepsin B, kolagenazu-2 (matriks metaloproteinaze MMP-8), želatinaze (MMP-9), dipeptidil peptidaze (DDP) 2 i (DDP) 3 i elastaze. Takođe, Elei i Cox su pokazali korelaciju između gingivalne dipeptidil peptidaze II i IV aktivnosti i parodontološkog gubitka pripoja kod bolesnika sa hroničnom parodontopatijom [26].

Aspartat aminotransferaza je citoplazmatski enzim koji se oslobađa iz nekrotičnih ćelija. On je identifikovan u gingivalnoj tečnosti, a povišeni nivoi su povezani sa periodontalnom destrukcijom tkiva. Oringer PJ i sar. pokazuju da su

retrospective analysis of GCF-PGE2 by examining the longitudinal relationship of PGE2 concentrations in GCF to attachment loss in adult patients with periodontitis. Results showed that elevated PGE2 was detectable in GCF 6 months before the identification of periodontal disease activity and significantly decreased 1 month after scaling and root planning was provided [20]. PGE2 acts as a potent vasodilator and increases capillary permeability, which elicits clinical signs of redness and edema. PGE2 also stimulates fibroblasts and osteoclasts to increase production of MMPs. Many clinical trials explored the use of a COX-2 inhibitor as an adjunct to periodontal therapy. These inhibitors improved the clinical outcome after periodontal therapy compared to periodontal therapy alone [21,22]. These studies indicate that prostaglandins, especially PGE-2, may be involved in periodontitis induced bone resorption. Ultimately, MMPs affect the remodeling and degradation of the periodontium Later, it was found that there is a correlation between increased PGE2 concentrations and clinical attachment loss in patients with moderate or advanced periodontal disease. Nevertheless, PGE2 has shown much promise as a biomarker of periodontitis.

Proinflammatory cytokines,particularly IL-1 beta, may play an important role in the etiology of periodontal disease. The severity of periodontitis is associated with local (GCF or tissue) increase in IL1-beta, TNF-alpha, and prostaglandins such as PGE2, whereas the inhibition of these substances produces a substantial reduction in periodontal disease. Liu et al. confirmed that with the increase of gingival index inflammation and increase in the depth of the periodontal pocket there is a corresponded increase in the concentration of IL1-beta both in the gingival fluid and also in the gingival tissue [23,24]. Stefanovska in her study indicates a significant increase in the concentrations of IL1-alpha and IL1-beta in the gingival fluid with an increase in the degree of gingival inflammation [25]. Loos and Toja [16] analyze the potential diagnostic markers in gingival fluid in patients with periodontitis. Their researches identify 8 potentially useful markers, including:

povišeni nivoi AST prisutni na mestima koji pokazuju parodontalnu progresiju bolesti. Visoka prevalentnost AST, pozitivna mesta zbog upale gingive, smanjuje sposobnosti testa da razlikuje progresivna i stabilna, ali i inflamirana mesta[27].

Alkalni fosfati su glikoproteini odgovorni za očuvanje alveolarne kosti i celokupni periodontalni ligament. Pretpostavlja se da je njihovo poreklo u gingivalnoj tečnosti prvenstveno iz polimorfonuklearnih leukocita. Smatra se da alkalna fosfataza igra ulogu u metabolizmu kosti i formiranju kolagena. Aktivnost alkalne fosfataze je u korelaciji sa dubinom parodontalnog džepa i procenatom gubitka kosti i nađeno je da je ova aktivnost 20 puta veća u gingivalnoj tečnosti kod inflamiranih regija nego u serumu. Slični nivoi alkalne fosfataze u gingivalnoj tečnosti otkriveni su u zdravoj i inflamiranoj gingivi, ali longitudinalne studije koje je sproveo Nakashima potvrđuju da su nivoi alkalne fosfataze veći kod inflamiranih regija[28].

Glukuronidaza koja se nalazi u azurofilnim ili primarnim granulama PMN-a vrši degradacijuprotoglikana i potpornihsupstanci. Ona je homeotetramer koji se sastoji od četiri identične podjedinice. Enzim se oslobađa iz makrofaga, fibroblasta i endotelijalnih ćelija zdrave ili hronično inflamirane gingive. Karakteriše se visokom osetljivošću i specifičnošću, koja se odnosi na mogućnost gubitka pripoja. Tako se ovaj enzim može javiti kao dobar prediktor odgovora na lečenje i procenu rizika od budućeg oštećenja parodontalnih tkiva [12].

Katepsin B je cistein proteaza uključen u proteolitička očuvanja. U gingivalnoj tečnosti makrofazi su glavni proizvođači katepsina B [29]. Kunimatsy K i sar.[30] su primetili da su nivoi katepsina B povišeni kod pacijenata sa parodontopatijom u odnosu na one sa inflamacijom gingive, uprkos sličnom načinu gingivalne eksudacije. Izvor katepsina B u tečnosti je uglavnom iz makrofaga i njegove analize su uvek različite kod pacijenata sa parodontopatijom i kod pacijenata sa hroničnom gingivalnom inflamacijom. Štaviše, nivoi tečnosti katepsina B su usko povezani sa kliničkim parametrima pre i posle tretmana parodontopatije, sobzirom na korisnost ovog

phatase, beta glukuronidase, cathepsin B, collagenase-2 (matrix metall-oproteinase MMP-8), gelatinase (MMP-9), dipeptidyl peptidase (DDP) 2 and (DDP) 3 and elastase. Also, Eley and Cox demonstrated the correlation between gingival crevicular fluid dipeptidyl peptidase II and IV activity and periodontal attachment loss in patients with chronic periodontitis [26].

Aspartate aminotransferase is a cytoplasmatic enzyme that is released from necrotic cells. It has been identified in gingival crevicular fluid (GCF), and elevated levels are associated with periodontal tissue destruction. Oringer RJ at al. demonstrates that elevated levels of AST were present at sites that did not subsequently exhibit periodontal disease progression. The high prevalence of AST-positive sites due to gingival inflammation diminished the test's ability to discriminate between progressive and stable, but inflamed sites [27].

Alkaline phosphates are glycoproteins responsible for the preservation of alveolar bone and replenishment of the periodontal ligament. It is assumed that thein origin in gingival fluid is primarily from polymorphonuclear leukocytes. Alkaline phosphatase is thought to play a role in bone metabolism and collagen formation. The activity of alkaline phosphatase has been shown to be correlated with pocket depth and the percentage of bone loss, and this activity was found to be 20 times higher greater in GCF from active sites than in serum. Similar levels of alkaline phosphatase in gingival fluid were detected in healthy and inflamed gingiva, but a longitudinal study conducted by the Nakashima confirms that the elevations of the level of alkaline phosphatase predate the clinical attachment loss and that the total quantity of alkaline phosphatase in fluid was significantly higher at affected sections[28].

Glukuronidase found in the azurophilic or primary granules of PMNs degrades proteoglycans and supporting substances. Its a homeotetramer composed of four identical subunits. The enzyme is liberated from macrophages, fibroblasts and endothelial cells of healthy or chronically inflamed gingiva. It shows a high sensitivity and specificity which is related to the possibility of clinical attachment loss. Thus this enzyme may occur as a good predictor of the response to the treatment and assessment of the risk of future periodontal impairment [12].

enzima u proceni rezultata lečenja. Elei i Cox analizirali su katepsin B i ocenili njegovu primenu u proceni nastanka kliničkog gubitka pripoja[31]. Četrdeset i devet pacijenata je bilo praćeno nakon inicijalne parodontološke terapije u trajanju od dve godine. Ukupno 121 mesta su pronađena sa kliničkim gubitkom pripoja (90 sa brzim gubitkom i 31 sa postepenim). Nivoi katepsina B bili su veći na mestima sa brzim gubitkom. Štaviše, na mestima sa postepenim gubitkom pripoja, nivoi katepsina B su bili povišeni u poređenju sa kontrolnimmestima. Katepsin B doprinosi uništenju parodoncijuma direktno ili indirektno, kroz proteolitičke aktivnosti latentne neutrofilne prokolagenaze (MMP-8) [32].

Matriks metaloproteinaze su enzimi izvedeni od ćelija domaćina, poreklom iz veoma važne porodice neutral-proteinaza, i učestvuju u regularnom ciklusu parodoncijuma kao i u destruktivnom procesu parodontopatije, koji može da se meri u gingivalnom tečnosti[33,34, 35]. Neutrofili su glavne ćelije odgovorne za oslobađanje MMP na bolesnim mestima, posebno MMP-8 (kolagenaze-2) i MMP-9 (želatinaza-B)[36]. MMP-8 je u stanju da potencijalno degradira tranzitivni kolagen, dok MMP-9 degradira nekoliko ekstracelularnih matriks proteina[37]. Pokazano je da se aktivnost kolagenaze povećava sa povećanjem težine zapaljenja, povećanjem dubine džepa i gubitkom alveolarne kosti [17]. Chen i sar.[38] su ustanovili povišen nivo aktivne neutrofilne kolagenaze u tečnosti gingive kod pacijenata sa parodontopatijom. Ovi aktivni oblici neutrofilne MMP-8 i MMP-13 u gingivalnoj tečnosti potvrđeni su kao učesnici u kolagenaznoj aktivnosti. Ostali MMP MMP-3 i TIMP-1 u gingivalnoj tečnosti ispitivani su kao prognostički faktori za napredovanje parodontopatije kod 40 sistemski zdravih osoba u periodu od šest meseci. Tečnost je sakupljena iz zdravih i obolelih delova svakog pacijenta. Prosečna količina MMP-3 i TIMP-1 u inflamiranim regijama bila je znatno veća u odnosu na zdrave regije. Nivo MMP-3 u tečnosti je bio u visokoj korelaciji sa kliničkim merenjima obavljenim na početku i posle tri i šest meseci. Sve ove regije sa visokim nivoom tečnosti iz MMP-3 i TIMP-1 su pod znatno većim rizikom od progresije paradontopatije.

Cathepsin B is a cysteine protease involved in the proteolytic preservation services. In gingival fluid, macrophages are the main producers of cathepsin B[29]. Kunimatsu K et al.[30] noticed that the levels of cathepsin B were elevated in patients with periodontal disease compared to those with gingival inflammation, despite the similar term of gingival flow. The source of cathepsin B in fluid is mainly from macrophages and its analyses in fluid expectancy is different in patients with periodontal disease and in patients with chronic gingival inflammation. Moreover, fluid levels of cathepsin B correlated significantly with clinical parameters before and after periodontal treatment, assuming the usefulness of this enzyme in the evaluation of the results of treatment. Eley and Cox studied cathepsin B and evaluated its use as a predictor of attachment loss[31]. Forty-nine patients were monitored after initial periodontal therapy for 2 years. A total of 121 sites were found with attachment loss (90 with rapid loss and 31 with gradual loss). Cathepsin B levels were higher at the sites with rapid loss than in the paired control sites. Moreover, at the sites with gradual attachment loss, cathepsin B levels were elevated when compared with the paired control sites. Cathepsin B aided in the destruction of periodontal tissue directly or indirectly, through the proteolytic activity of latent neutrophil procollagenase (promatrics metalloproteinase-8) [32].

Matrix metalloproteinase are host cell-derived enzymes, originating from a very important family of neutralproteinases, and participate in the regular cycle of periodontal tissue as well as in the destructive process of periodontal disease, that can be measured in GCF [33, 34, 35]. The neutrophils are the major cells responsible for MMP release at the infected site, specifically for MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) [36]. Although MMP-8 is able to potently degrade interstitial collagens, MMP-9 degrades several extracellular matrix proteins[37].GCF collagenase activity has been shown to increase with increasing severity of inflammation and increasing pocket depth and alveolar bone loss[17]. Chen *et al.* (38) established an elevated level of active neutrophil collagenase in gingival fluid in patients with periodontitis. These active

Neutrofilna elastaza je moćan proteolitički enzim detektovan u azurofilnim granulama PMN koji su analogni lizozomima. Deluje na elastin, proteoglikane, hemoglobin, fibrinogen i kolagen. Leukocitna elastaza degradira zrela kolagena vlakna. Dokazano je da se nivo elastaza u gingivalnoj tečnosti povećava kod izazvanog eksperimentalnog gingivitisa i smanjuje nakon uklanjanja zubnog plaka. Elei i Cox [39] su potvrdili da su povećani nivoi elastaze parametari periodontalnog gubitka. Dugogodišnja posmatranja odraslih pacijenata sa paradentozom, koji su izloženi terapiji, pokazala su pozitivne korelacije elastaza u gingivalnoj tečnosti sa kliničkom gubitkom pripoja. Dirjanska, takođe, potvrđuje povećani nivo elastaza kod pacijenata sa početnom periodontopatijom [40].

Potencijalni markeri parodont-opatije poreklom iz pljuvačke

Pljuvačka je još jedna značajna oralna tečnost. Pljuvačka je sekret pljuvačnih i mukoznih žlezda i veoma važna u održavanju oralnog zdravlja. Ona je lako dostupna za prikupljanje neinvazivnim metodama i sadrži lokalno proizvedene mikrobe i medijatore, isto kao i gingivalnu tečnost. Pljuvačka se takođe smatra korisnom za skrining parodontopatije i praćenje odgovora na tretman[41]. Za razliku od gingivalne tečnosti, kolekcija pljuvačke se tehnički obavlja lakše. Sastojci pljuvačke koji su proučavani kao potencijalni dijagnostički biomarkeri za paradentoze uglavnom uključuju proteine koji potiču iz domaćina (enzima i imunoglobulina, fenotipskih markera, ćelija domaćina, hormona, bakterija i bakterijskih proizvoda, jona i isparljivih komponenti)[42]. Pvljuvačka sadrži faktore koji potiču iz domaćina i bakterija, uključujući nekoliko enzima koji degradiraju proteine, proteoglikane, lipide i ugljene hidrate. Enzimi u pljuvački mogu poticati iz ćelija pljuvačnih glandula, mikroorganizama, epitelnih ćelija i PMN iz gingivalne tečnosti. Dipeptidilpeptidaza (DDP-IV) i alanin aminopeptidaza (AAP) su proteinaze koje učestvuju u degradaciji kolagena. Studija Elgun i sar.[43] ukazuje na povećanu aktivnost DDP –IV i AAP u pljuvački kod pacijenata sa parodontozom

forms of neutrophil MMP-8 and MMP-13 in gingival fluid have been confirmed as participants in collagenase activity. The other MMPs, MMP-3 and TIMP-1 in gingival fluid are evaluated as prognostic factors for the progression of the periodontal disease in 40 systemically healthy individuals over a six month period. Fluid was collected from healthy and affected sections of each patient. The average quantity of MMP-3 and TIMP-1 in the affected site was significantly higher in comparison to healthy sections. Fluid levels of MMP-3 highly correlated with clinical measurements performed at the beginning and after 3- and 6-month periods. All these sections with high fluid levels of MMP-3 and TIMP-1 are under significantly higher risk of the progression of periodontal disease.

Neutrophyl elastase is an aserine potent proteolytic enzyme detected in the azurophil granules of PMNs, which are analogues to lysozymes. It acts upon elastin, proteoglycans, haemoglobin, fibrinogen and collagen. Leukocyte elastase degrades mature collagen fibers. It has been proved that levels of elastase in gingival fluid increase by the induction of experimental gingivitis and are reduced after the removal of dental plaque. Eley and Cox [39] confirm that increased fluid levels of elastase are the precursor of periodontal attachment loss. Long-standing observations of adult patients with periodontal disease who were subjected to periodontal therapy have shown positive correlations of elastase in fluid with clinical attachment loss. Dirjanska also confirmed the increased fluid levels of elastase in patients with initial periodontal disease [40].

Possible salivary markers for periodontal disease

Another oral fluid that has recently gained significant recognition is saliva. Saliva is a secretion of the salivary and mucous glands and very important in maintaining oral health. It is quickly accessible for collection through a noninvasive method and contains local produced microbes and mediators of host response such as as gingival fluid. Saliva is also considered a useful medium fa screening periodontal disease and monitoring treatment response [41]. Unlike gingival fluid, the collection of saliva is technically performed more easily. Salivary

u odnosu na zdrave osobe. Neutrofili su važne ćelijske vrste u odgovoru domaćina na patogene bakterije. Neutrofilne granule sadrže hidrolitički neutralne enzime kao što su elastaza, katepsin G, mieloperoksidaze i lizozime, kao i hidrolaze, uključujući: katepsin B, katepsin D i glukuronidaze. Laktoferin i neutofil kolagenaze (MMP-8) i (MMP-9) se takođe čuvaju u neutrofilnim granulama.Uočeni su povišeni nivoi neutrofilnih proteina kao što su lizozimi, mieloperoksidaze i laktoferin kod periodontalnih oštećenja. Potvrđeno je da je laktoferin u interakciji sa Actinobacilus actinomocetem comitans, mikroorganizmom koji dovodi do agresivne forme parodontopatije [44].

U granulama neutrofila MMP su takođe povezani sa destrukcijom paradontalnog tkiva i otkriveni u pljuvački pacijenata [45]. Ostala rana ispitivanja salive su ukazala na značajno povećanje nivoa kolagenaze 2 (MMP-8) kod pacijenata [46,47]. Povišene MMP-8 nivoe u aktivnoj progresiji bolesti opisali su Lee i sar. u longitudinalnoj studiji pacijenata sa gingivitisom, bez progresivne i sa progresivnom parodontopatijom. Primećeno je da je ukupna aktivnost kolagenaze 50% veća u grupi sa progresijom bolesti[15]. Ristoska u svojoj studiji potvrđuje da je prosečna vrednost MMP-8 u pljuvački bila značajno veća kod pacijenata sa paradentozom nego u kontrolnoj grupi [48,49]. MMP-8 je potvrđen ne samo kao indikator bolesti, već i kao indikator njene aktivnosti [50]. MMP-8 može imati neku buduću vrednost kao dijagnostički marker za parodontopatiju, indikator za progresiju bolesti, ali i kao signal sa kojim se može utvrditi efikasnost lečenja [37].

Alkalna fosfataza (ALP) je enzim katalaze koji ubrzava uklanjanje fosfatnih grupa iz raznih molekula, uključujući nukleotide, proteine i alkaloide. Iako je prisutan u svim tkivima, ALP je naročito koncentrisan u kostima, jetri, žučnim putevima, bubrezima i placenti. Od značaja za oralno zdravlje je, naravno, povezanost između ALP-a i parodontopatije [51]. Rana istraživanja ALP i parodontopatije u eksperimentalnom modelu gingivitisa su pokazala značajnu korelaciju između ALP i dubine parodontalnih džepova i između ALP i težine inflamcije [52]. Nakamura i Slots su

constituents which are studied as potential diagnostic biomarkers for periodontal disease mainly include proteins originating from the host (enzymes and immune globulin, phenotypic markers, host cells, hormones, bacteria and bacterial products, ions and volatile components) [42]. Saliva contains factors originating from the host and bacteria, including several enzymes that degrade proteins, proteoglycans, lipids and carbohydrates. Enzymes in saliva can originate from cells in salivary glandules, microorganisms, epithelial cells, and PMN from gingival fluid. Dipeptidilpeptidaza (DDP-IV) and alanine aminopeptidase (AAP) are proteinases that participate in collagen degradation. In the study by Elgun et al. [43] increased activity of DDP –IV) was verified, as well as AAP in saliva in patients with periodontal disease compared to healthy ones.

Neutrophils are important cellular types in the host response to pathogenic bacteria. Neutrophil granules contain hydrolytic neutral enzymes such as elastase, cathepsin G, myeloperoxidases and lysozyme, as well as hydrolases, including: cathepsin B, cathepsin D and Glucuronidase. Lactopherin, neutophil collagenase (MMP-8) and (MMP-9) are also stored in neutrophil granules. Neutrophil proteins such as lysozymes, myeloperoxidases and lactopheryn are noted as elevated levels in a state of periodontal destruction. It has been confirmed that the lactopheryn is in interaction with Actinobacilus actinomyce temcomitans, which represents a causative microorganism for the aggressive form of periodontal disease [44].

MMPs in neutrophil granules are also associated with periodontal tissue destruction and are detected in saliva in patients with periodontal disease [45]. Other early saliva-based investigations detected significantly increased levels of collagenase 2 (MMP-8) in periodontal disease patients [46,47]. Elevated MMP-8 levels in active disease progression were observed by Lee et al. in a longitudinal study using patients with gingivitis, no progressive and progressive periodontitis. The total collagenase activity was observed to be 50% higher in the disease progression group [15]. Ristoska in her study confirms tha the average value of MMP-8in saliva was significantly higher in patients with periodontal disease than the control group[48,49]. MMP-8 was confirmed not only

proučavali ukupno 76 enzima u mešovitoj pljuvački i potvrdili veću aktivnost enzima kod osoba sa parodontozom nego kod zdravih pojedinaca [53]. Analizirani su uzorci pljuvačke pacijenata sa parodontozom i otkriveno je da su periodontalna oštećenja povezana sa višim nivoima ALP-a u pljuvački. Ove rezultate potvrđuju i zaključci Todorovića i dr. koji ukazuju da se povećana aktivnost pljuvačne ALP vidi kod pacijenata sa parodontopatijom u odnosu na zdrave u kontrolnoj grupi [54].

Imunoglobulini mogu uticati na oralnu mikrofloru sprečavanjem bakterijskog metabolizma. Ig-, Ig-G Ig-M a potvrđene su veće koncentracije kod pacijenata sa parodontozom u poređenju sa zdravim pacijentima [55,42,56]. Naknadna istraživanja imunoglobulina u pljuvački, a u vezi sa periodontalnim patogenima, ukazala su na izvesne korelacije sa statusom parodontopatije.

C-reaktivni protein (CRP) je sistemski marker koji se oslobađa tokom akutne faze inflamatornog odgovora, a proizvodi ga lien, sprovodeći razmenu gingivalne tečnosti u pljuvački kroz tečnosti gingive ili pljuvačne žlezde. Visoki nivoi CRP-a su povezani sa hroničnom ili agresivnom parodontopatijom [57]. Peterson i sar. su merili količine katepsina G, elastaze, inhibitora elastaze i C-reaktivnog proteina (CRPS) i utvrdili su da su njihovi nivoi direktno povezani sa periodontalnim statusom pojedinca. Prema parodontološkom statusu klasifikovano je četrdeset pet učesnika (zdrav, gingivitis, blaga do umerena parodontopatija, ili umerena do ozbiljna parodontopatija) i prikupljen je uzorak pljuvačke. Sa izuzetkom Alfa 1-antitripsina, povećani su bili svi pljuvačni nivoi ciljanih markera sa povećanjem težine bolesti [58, 59].

Može se shvatiti iz svih ovih studija da se nekoliko medijatora pljuvačke pominje u patogenezi parodontopatije. Kako je pljuvačka neinvazivni medijum koji se češće ispituje, postoje mogućnosti da se kao biološka tečnost analizira zbog postavljanja dijagnoze parodontopatije. Pljuvačka može pojedinačno da se prikuplja i nudi profitabilni pristup za skrining većih populacija. Ipak, postoji potreba za dobro kontrolisanim kliničkim studijama koje bi se sprovele u opštoj populaciji, kako bi se potvrdio značaj specifičnih dijagnostičkih markera.

as an indicator of disease, but also as an indicator of the disease activity [50]. MMP-8 may have some future value as a diagnostic marker for periodontal disease, an indicator for disease progression, and as a signal to determine the efficacy of treatment [37].

Alkaline phosphatase (ALP) is a catalyzing enzyme that accelerates the removal of phosphate groups from a variety of molecules, including nucleotides, proteins, and alkaloids. Although present in all tissues, ALP is particularly concentrated in the bone, liver, bile duct, kidney, and placenta. Of interest for the in oral health, of course, is the association between ALP and periodontal disease [51]. Early investigations of ALP and periodontal disease in an experimental gingivitis model showed a significant correlation between ALP and pocket depth and between ALP and inflammation[52]. Nakamura and Slots studied a total of 76 enzyme activities in mixed whole saliva and noted higher enzyme activity in individuals with periodontal disease than non-diseased individuals[53]. Salivary samples from patients with confirmed periodontal disease were analyzed and revealed that periodontal destruction by measurement of probing depth, gingival bleeding, and suppuration were related to higher ALP levels in saliva. These results are supported findings of Todorovic *et al*. which demonstrate that increased activity of salivary ALP is seen in patients with periodontal disease in relation to a non-diseased control group [54].

Immunoglobulins have an effect on oral microflora in a way that prevents adherence and bacterial metabolism, and confirms that salivary Ig-A, Ig G Ig-M had higher concentrations in patients with periodontal disease compared with healthy patients [42,55,56]. Subsequent explorations of immunoglobulins found in whole saliva directed against periodontal pathogens have indicated some correlations with the status of periodontal disease.

C-reactive protein (CRP) is a systemic marker which is released during the acute phase of inflammatory response and is produced by lien, circulating CRP exchange in saliva through gingival fluid or salivary glandules. High levels of CRP are associated with chronic or aggressive periodonta disease[56]. Peterson et al. measured quantities of host-response indicators cathepsin G,l

Zaključak

U poslednje dve decenije prisutan je uzlazni trend u razvoju sredstava za praćenje parodontopatije. Istovremeno, promovisani su novi biomarkeri u razvoju novih terapeutskih dostignuća.

Uprkos činjenici da, izazovi ostaju, oralni dijagnostičke biomarkeri pružaju nadu za njihovu primenu u dijagnostici i prognozi parodontopatije i, na taj način, nova paradigma parodontološke dijagnoze bi na kraju uticala na poboljšanje kliničkog lečenja. Danas nijedan marker iz gingivalne tečnosti ili kombinacija markera ne može da utvrdi da li je periodontalni tretman dovoljan da spreči parodontalnu destrukciju. Neki od enzima domaćina i produkti raspadanja tkiva su najperspektivniji potencijal markera gingivalne tečnosti za prognozu bolesti, posebno onih koje degradiraju kost. Buduća istraživanja ovih markera će obezbediti obećavajuće rezultate.

elastase, elastase inhibitors, and C-reactive proteins (CRPs) - to determine whether their levels were directly related to the individual's periodontal status. Forty-five participants were categorized according to periodontal status (healthy, gingivitis, mild-to-moderate periodontitis, or moderate-to-severe periodontitis) and whole-saliva samples were collected. With the exception of alpha 1-antitrypsin, an increase in salivary levels for all of the targeted host-response markers correlated with increasing severity of disease [57, 58].It can be understood from all these studies that several mediators of saliva are mentioned in the pathogenesis of periodontal disease. Whereas saliva is a non-invasive medium and is examined more frequently, it gives significant promise as a biological fluid which can be analyzed for the diagnosis of periodontal disease. Saliva can be individually collected and can offer a profitable approach for the screening of larger populations. Nevertheless, there is a need for well-controlled clinical studies conducted in the general population in order to confirm the significance of the specific diagnostic markers.

Conclusion

In the last two decades there has been an equitable upward trend in the development of the means of monitoring of periodontal disease. At the same time, new biomarkers are promoted in the development of new therapeutic achievements.

Despite the fact that challenges remain ahead, oral diagnostic biomarkers give hope for their applicative use in the diagnosis and prognosis of periodontal disease and, in that way, a new paradigm for periodontal diagnosis would ultimately affect improved clinical management of periodontal patients. Today, no single GCF marker or combination of GCF markers is available to determine whether periodontal treatment is sufficient and⁄ or necessary to prevent further periodontal breakdown. Some of the host tissue enzymes and tissue breakdown products are the most promising potential GCF markers of disease progression, especially of those diseases that degrade the bone. Future research on these bone markers will provide promising results.

LITERATURA / REFERENCES

1. Savage A, Eaton KA. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. J Clin Periodontol 2009; 36:458–467.
2. Taubman MA, Kawai T, Han X. The new concept of periodontal disease pathogenesis requires new and novel therapeutic strategies. J Clin Periodontol 2007; 34(5):367-369.
3. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. J Clin Periodontol 2011; 38(Suppl 11):85–105.
4. Kinane DF. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. Periodontology 2000; 24: 215–225.
5. Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective [review]. Periodontol 2000 1994; 5:7-25.
6. Miyasaki, KT, Nisengard RJ, Haake SK. Immunity and inflammation; basic concepts, in: Newman MG, Takei HH, Carranza FA (Eds.), Carranza's Clinical Periodontology. W.B. Saunders, Philadelphia 2004; pp. 113– 132.
7. Armitage GC. The complete periodontal examination. Periodontol 2000 2004; 34:22-33.
8. Colburn WA. Biomarkers in drug discovery and development: from target identification through drug marketing. J ClinPharmacol 2003; 43(4):329-341.
9. Beklen A, Ainola M, Hukkanen M, Gürgan C, Sorsa T, Konttinen YT. MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. J Dent Res 2007; 86 (4):347–351.
10. Ozmeric N. Advances in periodontal disease markers. Clin Chim Acta 2004; 343:1-16.
11. Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid markers as predictors of risk for periodontal diseases. Periodontology 2000 2003; 31:167–180.
12. Lamster IB, Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases [review]. Ann N Y Acad Sci 2007; 1098:216-229.
13. Sanders AE, Slade GD, Fitzsimmons TR. Physical activity, inflammatory biomarkers in gingival crevicular fluid and periodontitis. J Clin Periodontol 2009; 36:388–395.
14. Armitage GC, 2004. Analysis of gingival crevice fluid and risk of progression of periodontitis. Periodontology 2000 2004; 34:109–119.
15. Lee W, Aitken S, Sodek J, Mc Culloch CA. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. J Periodontal Res 1995; 30:23-33.
16. Loos BG, Tjoa S. Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? Periodontology 2000 2005; 39:53-72.
17. Villela B, Cogen RB, Bartolucci AA, Birkedal-Hansen H. Collagenolytic activity in crevicularfluid from patients with chronic adultperiodontitis, localized juvenileperiodontitis and gingivitis, and fromhealthy control subjects. J Periodontal Res 1987; 22(5):381-389.
18. Airila-Mansson S, Soder B, Kari K, Meurman JH. Influence of combinations of bacteria on the levels of prostaglandin E2, interleukin-1beta, and granulocyte elastase in gingival crevicular fluid and on the severity of periodontal disease. J Periodontol 2006; 77:1025-1031.
19. Choi BK, Moon SY, Cha JH, et al. Prostaglandin E (2) is a main mediator in receptor activator of nuclear factor-kappa B ligand-dependent osteoclastogenesis induced by Porphyromonas gingivalis, Treponema denticola, and Treponema socranskii. J Periodontol 2005; 76(5):813-820.
20. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodontal Res 1986; 21:101-112.
21. Pinho MDN, Pereira LB, Souza SLE et al. Short-term effect of COX-2 selective inhibitor as an adjunct for the treatment of periodontal disease-a clinical double-blind study in humans. Braz Dent J 2008; 19(4):323–328.
22. Alec Yen C, Damoulis PD, Stark PC, Hibberd PL, Singh M, Papas AS. The effect of a selective cyclooxygenase-2 inhibitor (celecoxib) on chronic periodontitis. J Periodontol 2008; 79(1):104–113.
23. Liu CM, Hou LT, Wong MY, Rossomando EF. Relationships between clinical parameters, interleukin 1B and histopathologic findings of gingival tissue in periodontitis patients. Cytokine 1996; 8:161-167.
24. Kamma J, Mombelli A, Tsinidou K. Cytokines in gingival crevicular fluid of adolescents and young adults. Oral Microbiol Immunol 2009; 24:7–10.
25. Stefanovska E. Inflammatory cytokines and their role in modulation of the clinical expression at plaque-induced gingival inflammation. PhD dissertation, Skopje, 2011.
26. Eley BM, Cox SW. Correlation between gingival crevicular fluid dipeptidyl peptidase II and IV activity and periodontal attachment loss. A 2-year longitudinal study in chronic periodontitis patients. Oral Dis 1995; 1(4):201-213.
27. Oringer RJ, Howell TH, Nevins ML, Reasner DS, Davis GH, Sekler J *et al*. Relationship between crevicular aspartate aminotransferase levels and periodontal disease progression. J Periodontol 2001; 72(1):17-24.
28. Nakashima K, Roehric N, Cimasoni G. Osteocalcin, prostaglandin E-2 and alkaline phosphatase in gingival cervicular fluid: their relations to periodontal status. J Clin Periodontol 1994; 21(5):327-333.
29. Kennett CN, Cox SW, Eley BM. Investigations into the cellular contribution to host tissue proteases and inhibitors in gingival crevicular fluid. J Clin Periodontol 1997; 24(6):424-431.
30. Kunimatsu K, Ichimaru E, Kato I. Granulocyte medullas in levels in gingival crevicular fluid

from chronic adult periodontitis patients and experimental gingivitis subjects. J Periodontal Res 1990; 25:352–357.
31. Eley BM, Cox SW. The relationship between gingival crevicular fluid cathepsin B activity and periodontal attachment loss in chronic periodontitis patients: a 2-year longitudinal study. J Periodontal Res 1996;31:381-392.
32. Garima Garg, Manoj Kumar Thorat, A. R. Pradeep, Happy Daisy, Parag Hadge. Correlation of gingival crevicular fluid levels of cathepsin K and oncostatin M in periodontal health and disease. AOSR 2012; 2(2):60-66.
33. Gapski R, Barr JL, Sarment DP, et al. Effect of systemic matrix metalloproteinase inhibition on periodontal wound repair: a proof of concept trial. J Periodontol 2004; 75(3):441-452.
34. Makela M, Salo T, Uitto VJ, *et al*. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J Dent Res 1994; 73(8):1397-1406.
35. Söder B, Jin LJ, Wickholm S. Granulocyte elastase, matrixmetalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. J Clin Periodontol 2002; 29(5):384-391.
36. Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H, Salo T et al. Gingival crevicular fluid collagenase2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 2003; 38(4):436-439.
37. Ryan ME, Ramamurthy S, Golub LM. Matrix metalloproteinases and their inhibition in periodontal treatment. Curr Opin Periodontol 1996; 3:85-96.
38. Chen HY, Cox SW, Eley BM, Mantyla P, Ronka H, Sorsa T. Matrix metalloproteinase-8 levels and elastase activities in gingival crevicular fluid from chronic adult periodontitis patients. J Clin Periodontol 2000; 27:366-369.
39. Eley BM, Cox SW. A 2-year longitudinal study of elastase in human gingival crevicular fluid and periodontal attachment loss. J Clin Periodontol 1996; 23:681-692.
40. Dirjanska K. Clinical and biochemical detection of initial periodontal lesion. Master's thesis, Faculty of Dentistry, Skopje, 2002.
41. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis 2002; 8:69-76.
42. Hagewald S, Bernimoulin JP, Kottgen E, Kage A. Salivary IgA subclasses and bacteria-reactive IgA in patients with aggressive periodontitis. J Periodontal Res 2002; 37: 333–339.
43. Elgun S, Ozmeric N, Demirtas S. Alanine aminopeptidase and dipeptidylpeptidase IV in saliva: the possible role in periodontal disease. Clinica Chimica Acta 2000; 298:187-91.
44. Alugupalli K, Kalfas S, Edwardsson S, Naidu A. Lactoferrin interaction with Actinobacillus actinomycetemcomitans. Oral Microbiol Immunol 1995; 10:35-41.
45. Rayyan A. Kayal. The Role of Osteoimmunology in Periodontal Disease. Bio Med Research International 2013, Article ID 639368, 12 pages.
46. Iijima K, Ando K, Kishi M, et al. Collagenase activity in human saliva. J Dent Res 1983; 62:709–712.
47. Sorsa T, Hernández M, Leppilahti J, Munjal S, Netuschil L, Mäntylä P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. Oral Dis 2010; 16(1):39–45.
48. Ristoska S. Salivary matrix-metaloproteinase 8 (MMP-8), biomarker of the periodontal activity. Macedonian Dental Review 2008; 32 (3-4):173-180.
49. Kushlinskii NE, Solovykh EA, Karaoglanova TB, Bayar U, Gershtein ES, Troshin AA, et al. Content of matrix metalloproteinase-8 and matrix metalloproteinase-9 in oral fluid of patients with chronic generalized periodontitis. Bull Exp Biol Med 2011; 152(2):240–244.
50. Konopka L, Pietrzak A, Brzezinska-Blaszczyk E. Effect of scaling and root planing on interleukin-1ß, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. J Periodontal Res 2012; 47(6):681–688.
51. Malhotra R, Grover V, Kapoor A, Kapur R. Alkaline phosphatase as a periodontal disease marker. Indian J Dent Res 2010; 21:531-536.
52. Ishikawa I, Cimasoni G. Alkaline phosphatase in human gingival fluid and its relation to periodontitis. Arch Oral Biol 1970; 15:1401-1404.
53. Nakamura M, Slots J. Salivary enzymes. Origin and relationship to periodontal disease. J Periodontol Res 1983; 18:559-569.
54. Todorovic T, Dozic I, Vicente-Barrero M, et al. Salivary enzymes and periodontal disease. Med Oral Patol Oral Cir Bucal 2006; 11:E115–E119.
55. Butchibabu K, Swaminathan M, Kumar S, Koppolu P, Kiran K, Muralikrishna T. Estimation of salivary immunoglobulin A levels in gingivitis and chronic periodontitis patients before and after phase I periodontal therapy. JNTR Univ Health Sci 2014; 3:23-27.
56. Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. J Period Res 2004; 39: 236-241.
57. Pederson ED, Stanke SR, Whitener SJ, et al. Salivary levels of alpha 2-macroglobulin, alpha 1-antitrypsin, C-reactive protein, cathepsin G and elastase in humans with or without destructive periodontal disease. Arch Oral Biol 1995; 40:1151-1155.
58. Haba D, Teslaru S, Ungureanu D, Hodorog D, Alecu C, Benghiac AG, Zetu L, Ancuţa C, Ancuţa E, Nemţoi A, Iordache C. Evaluation of serum and gingival crevicular fluid C-reactive protein and IL-6 levels in patients with periodontitis and transient ischemic attacks. Rom J Morphol Embryol 2011; 52(4):1243-1247.