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## Debljina intime-medije karotidnih arterija kod pacijenata s kroničnim i agresivnim parodontitisom

### *Carotid Intima-Media Thickness in Patients with Chronic and Aggressive Periodontitis*

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#### Sažetak

**Svrha:** Željela se istražiti povezanost debljine intime-medije (IMT) karotidnih arterija i parodontnog statusa kod pacijenata s parodontitisom. **Materijali i metode:** Istraživanje je provedeno na 128 ispitanika podijeljenih u četiri skupine. Svaka se sastojala od 37 pacijenata s kroničnim parodontitisom (18 muškaraca i 19 žena prosječne dobi 57,2±8,0 godina) i njih 30 s agresivnim parodontitisom (13 muškaraca i 17 žena prosječne dobi 34,7±6,3 godina). U kontrolnoj skupini za kronični parodontitis bilo je 27 ispitanika (11 muškaraca i 16 žena prosječne dobi 53,0±10,1 godina), a u onoj kontrolnoj za agresivni parodontitis 34 (15 muškaraca i 19 žena prosječne dobi 27,7±5,7 godina). U objema kontrolnim skupinama bili su parodontološki zdravi dobrovoljci. Stanje parodonta ocjenjivalo se parodontnim indeksima. U serumu su određeni trigliceridi, kolesterol HDL i LDL, ukupan serumski serum-kolesterol, visoko osjetljiv C-reaktivni protein (hsCRP) i glukoza. Mjerenje vrijednosti IMT-a na zajedničkoj karotidnoj arteriji obavljeno s pomoću Aloka ProSound ALPHA 10 s linearnom sondom od 13 MHz. **Rezultati:** U ovom istraživanju dobivene su značajno veće ukupne vrijednosti IMT-a (0,8 mm) i veće vrijednosti IMT-a desne karotidne arterije (0,8 mm) samo kod pacijenata s kroničnim parodontitisom. Kronični parodontitis bio je statistički značajan prediktor ( $p=0,003$ ) za vrijednosti IMT-a desne karotidne arterije u multivarijatnom modelu kada su u model bili uključeni ostali čimbenici rizika. U objema skupinama s parodontitisom bili su uglavnom nepušači te su dobivene statistički povećane vrijednosti hsCRP-a (2,2 i 1,4 mg/L). **Zaključak:** Istraživanje je pokazalo da kronični parodontitis može biti povezan s aterosklerozom karotidnih arterija, ali potrebna su dodatna istraživanja kako bi se preciznije odredio utjecaj agresivnog parodontitisa na vrijednosti IMT-a karotidnih arterija.

**Zaprimljen:** 19. kolovoza 2012.

**Prihvaćen:** 10. listopada 2012.

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#### Ključne riječi

parodontitis; parodontalni indeks; suženje karotide; tunika intima; C-reaktivni protein

#### Uvod

Parodontitis je kronična upalna bolest potpornih tkiva zuba koja nastaje kao odgovor na subgingivnu infekciju i rezultira progresivnim produbljanjem gingivnog sulkusa, destrukcijom alveolarne kosti te na kraju gubitkom zuba (1). Posljednjih je desetljeća znanstveno dokumentirana povezanost parodontne infekcije i sistemskih bolesti. Istraživanja su pokazala da parodontitis predstavlja neovisni rizični čimbenik za neželjene ishode trudnoće (2), lošu metaboličku kontrolu šećerne bolesti (3), respiratorne bolesti (4) i aterosklerozu s popratnim vaskularnim i tromboembolijskim komplikacijama (5).

Ateroskleroza je upalna bolest koja zahvaća sve slojeve arterija, a karakteriziraju je oštećenje stijenke krvne žile, smanjena permeabilnost te posljedično formiranje krvnog

#### Introduction

Periodontitis is a chronic inflammatory disease of the tooth supporting tissues a reaction to subgingival infection that leads to a progressive deepening of the gingival sulcus, alveolar bone destruction, and ultimately tooth loss (1). Over the past decades, a relationship between periodontal infection and systemic diseases has been scientifically documented. Studies have shown that periodontitis represents an independent risk factor for adverse pregnancy outcomes (2), poor metabolic control in diabetes (3), respiratory disease (4), and atherosclerosis with subsequent vascular and thromboembolic events (5).

Atherosclerosis is an inflammatory disease that affects all components of the arterial wall, and is characterized by vascular wall damage, reduction in vascular permeability, and

ugruška i tromboza (6). Parodontopatogene bakterije ulaze u sistemsku cirkulaciju kroz ulcerirani epitel parodontnog džepa koji kod pacijenata s parodontitisom može zauzimati površinu do 20 cm<sup>2</sup> (7). Ustanovljeno je da se tranzitorna bakterijemija pojavljuje nakon oralne higijene i struganja površine korijena, te čak poslije žvakanja (8). Koristeći se lančanom reakcijom polimeraze (PCR) u mnogobrojnim je istraživanjima, u uzorcima endarterektomije, identificiran DNK parodontnih bakterija. Najčešće se u aterosklerotičnim lezijama ispitivala bakterija *Porphyromonas gingivalis* koja može potaknuti prokoagulantni odgovor endotelnih stanica i nakupljanje LDL-kolesterola te tako olakšati stvaranje ateromatnog plaka (9,10).

Debljina intime-medije (IMT) definirana je uzorkom dviju linija koje se longitudinalno prikazuju ehotomografijom na objema stijenkama zajedničkih karotidnih arterija (CCA). Nalazi se između sučelja lumen-intima i medija-adventicija. B-mod ultrazvučno mjerenje vrijednosti IMT-a osnovno je sredstvo za određivanje najranijih stadija karotidne ateroskleroze. Ta neinvazivna tehnika prikazuje strukturu krvnih žila u većoj rezoluciji u usporedbi sa sličnim tehnikama (11). Gornja granica za karotidni IMT kod zdravih pojedinaca u dobi od 35 do 39, 40 do 49, 50 do 59 i 60 ili više godina procijenjena je na 0,60, 0,64, 0,71 i 0,81 milimetar (12). Iako su vrijednosti IMT-a povezane s dobi, obično se vrijednosti od >1,0 milimetra smatraju patološkim kod zdravih odraslih osoba te se često koriste kao granične u kliničkim istraživanjima ateroskleroze (13).

Svrha ovog rada bila je istražiti povezanost parodontnog statusa i vrijednosti IMT-a kod pacijenata s parodontitisom u usporedbi s parodontno zdravim ispitanicima. U analizu su uključeni i zajednički rizični čimbenici kako bi se dodatno potvrdila ta povezanost.

## Materijali i metode

Istraživanje je provedeno na 128 ispitanika podijeljenih u dvije ispitivane i dvije kontrolne skupine. Svi sudionici izabrani su u Zavodu za parodontologiju Stomatološkog fakulteta i Zavodu za neurologiju Kliničkog bolničkog centra *Seštara milosrdnica* u Zagrebu. Ispitivane skupine sastojale su se od 37 pacijenata s kroničnim parodontitisom (18 muškaraca i 19 žena prosječne dobi 57,2±8,0 godina) i njih 30 s agresivnim parodontitisom (13 muškaraca i 17 žena prosječne dobi 34,7±6,3 godina). U kontrolnoj skupini za kronični parodontitis bilo je 27 ispitanika (11 muškaraca i 16 žena prosječne dobi 53,0±10,1 godina), a u kontrolnoj za agresivni parodontitis 34 (15 muškaraca i 19 žena prosječne dobi 27,7±5,7 godina).

Kriteriji za uključivanje u ispitivanu skupinu bili su generalizirani umjereni do uznapredovali parodontitis i minimalno 20 preostalih zuba. Gubitak kliničkog pričvrstka od ≥3 mm na ≥30 posto mjesta korišten je za definiranje generaliziranih oblika parodontitisa. Kriteriji za isključivanje bili su sljedeći: nekontrolirane sistemske bolesti, nedavne akutne ili kronične infekcije, trudnoća, polifarmacija, prethodna parodontološka obrada, uzimanje sistemskih antibiotika u posljednjih šest mjeseci, teški pušači te ovisnost o alkoholu ili

eventually blood clot formation and thrombosis (6). Periodontal bacteria enter into the systemic circulation through the ulcerated epithelium of the periodontal pocket, which is a surface calculated to amount up to 20 cm<sup>2</sup> in periodontitis patients (7). It was found that transient bacteremia may occur after daily oral hygiene practices, scaling or even after chewing (8). Using polymerase chain reaction (PCR), there has been evidence identifying bacterial DNA of periodontal origin in human endarterectomy samples. The most investigated bacteria within atherosclerotic lesions was *Porphyromonas gingivalis*, that can induce a procoagulant response in endothelial cells, promote aggregation of LDL cholesterol thus facilitating atheromatous plaque formation (9, 10).

Intima-media thickness (IMT) is defined as a double-line pattern visualized by echotomography on both walls of the common carotid arteries (CCA) in a longitudinal image. It is localized between the lumen-intima and media-adventitia interfaces. B-mode ultrasound measurements of the IMT represent an established tool to quantify even early stages of carotid atherosclerosis. This noninvasive technique visualizes the wall structure with higher resolution than any other similar technique (11). The upper limits of carotid IMT for healthy individuals aged 35 to 39, 40 to 49, 50 to 59, and 60 years or older were estimated to 0.60, 0.64, 0.71, and 0.81 mm respectively (12). Although age-related, IMT values >1.0 mm are usually regarded as abnormal in healthy adults, and are often used as a threshold in clinical atherosclerosis trials (13).

The aim of this research was to investigate the relationship between periodontal condition and IMT in periodontitis patients, compared with periodontally healthy subjects. Common risk factors have been included in our analysis in order to further identify this relationship.

## Materials and methods

The study included 128 participants divided into two test and two control groups. All participants were recruited at the Department of Periodontology, School of Dental Medicine, and Department of Neurology, University Hospital Center "Sisters of Mercy" in Zagreb. The test groups consisted of 37 patients with chronic periodontitis (18 men and 19 women, mean age 57.2±8.0 years) and 30 patients with aggressive periodontitis (13 men and 17 women, mean age 34.7±6.3 years). There were 27 subjects in the control group for chronic periodontitis (11 men and 16 women, mean age 53.0±10.1 years), and 34 subjects in the control group for aggressive periodontitis (15 men and 19 women, mean age 27.7±5.7 years).

The inclusion criteria for test groups included generalized moderate to severe periodontitis and at least 20 remaining teeth. The presence of clinical attachment loss ≥3 mm at ≥30% of sites was used to define generalized forms of periodontitis. The exclusion criteria were uncontrolled systemic diseases, recent acute or chronic infections, pregnancy, long-term medication, previous periodontal treatment, systemic antibiotic therapy within the last 6 months, severe smoking, and alcohol or drug abuse. The control groups consisted of

drogama. Kontrolna skupina sastojala se od dobrovoljaca bez znakova parodontne bolesti. Pacijenti i kontrole nasumce su odabrani kako bi se izbjegla pristranost u selekciji i osigurala normalna raspodjela promatranih varijabli. Istraživanje je odobrilo Etičko povjerenstvo Stomatološkog fakulteta i provedeno je u skladu s etičkim načelima Helsinške deklaracije. Svi ispitanici potanko su informirani o istraživanju, što su potvrdili potpisivanjem pristanka prije kliničkog pregleda. Ispunili su i detaljni upitnik o svojoj medicinskoj anamnezi. Zabilježeni su sljedeći podaci: dob, spol, visina, težina, pušenje, uzimanje lijekova, te stupanj edukacije i fizičke aktivnosti.

Pregled parodonta kod svih je ispitanika obavio isti iskusni istraživač koji je izmjerio indeks plaka (PI), krvarenje pri sondiranju (BoP), dubinu sondiranja (PD), recesiju gingive (RE) i razinu kliničkog pričvrstka (CAL). Parodontni indeksi određivali su se standardnom parodontnom sondom (PCP-15, Hu-Friedy, Chicago, IL, SAD) i stomatološkim zrcalom. Vrijednosti IMT-a na CCA bilateralno određivale su se ultrazvukom (Aloka ProSound ALPHA 10, Tokio, Japan) s linearnom sondom od 13 MHz. Mjerenja je obavljao iskusni istraživač koji nije znao kojoj skupini pripada pojedini ispitanik. Krvni tlak u mirovanju izmjeren je prije ultrazvučnog pregleda. U serumu su određeni trigliceridi, kolesteroli HDL i LDL, ukupan serumski serum-kolesterol, visoko osjetljivi C-reaktivni protein (hsCRP) i glukoza.

Podaci su analizirani statističkim programom SPSS v.17.0 (SPSS Inc., Chicago, IL, SAD). Normalnost raspodjele varijabli provjeravala se Kolmogorov – Smirnovim testom. U statističkoj analizi kontinuiranih varijabli rabljeni su Studentov t-test i Mann-Whitneyjev U-test. Provedena je multivarijantna linearna regresijska analiza kako bi se ispitala statistička značajnost i doprinos CAL-varijable u objašnjavanju varijance IMT-a kada se u model uključe dob, spol, stručna sprema, fizička aktivnost, pušenje, indeks tjelesne mase (BMI), krvni tlak, glukoza, ukupni kolesterol, kolesteroli HDL i LDL, trigliceridi i hsCRP. Razina statističke značajnosti postavljena je na  $p < 0,05$ .

## Rezultati

Tijekom istraživanja pregledano je 57 (44,5%) muških i 71 (55,5%) ženskih ispitanika podijeljenih u četiri skupine.

Najviše pacijenata s kroničnim parodontitisom imalo je srednju stručnu spremu (64,9 %) i nisu se redovito bavili fizičkom aktivnošću (56,8%), dok su njihove kontrolne skupine bile visokoobrazovane (63,0 %) i bavile su se fizičkom aktivnošću barem jedanput ili dva puta na tjedan (74,1 %). U objema skupinama bili su uglavnom nepušači (59,5% i 55,6%). Značajne razlike nisu nađene za sljedeće vrijednosti: BMI, krvni tlak, trigliceride, HDL i LDL kolesterole, ukupni serumski kolesterol i glukoza. U odnosu na kontrolnu skupinu nađene su statistički veće vrijednosti hsCRP-a u ispitanjanoj skupini (2,2 i 0,7 mg/L, Mann-Whitneyjev test,  $p < 0,005$ ). Skupine su se statistički značajno razlikovale prema svim parodontnim indeksima ( $p < 0,001$ ; Mann-Whitneyjev test). Medijan PD i vrijednosti CAL-a kod pacijenata

volunteers with no signs of periodontal disease. The patients and the controls were randomly chosen to avoid selection bias and to ensure normal distribution of the sampled variables. This study was approved by the Ethics Committee of the School of Dental Medicine, and it was conducted in accordance with the ethical principles of the Helsinki Declaration. All participants were informed about the nature of the study and gave their written consent prior to the clinical examination. The subjects filled in an extensive questionnaire concerning their medical history. Their age, gender, height, weight, smoking habits, medication, educational level, and physical activity were recorded.

Periodontal examinations were performed in all subjects by one and the same experienced dental examiner, and included the assessment of plaque index (PI), bleeding on probing (BoP), probing depth (PD), gingival recession (RE) and clinical attachment level (CAL). The periodontal indices were taken using a standard periodontal probe (PCP-15, Hu-Friedy, Chicago, IL, USA), and dental mirror. IMT measurements on CCA were taken bilaterally by the same experienced and blinded sonographer, using an ultrasonograph (Aloka ProSound ALPHA 10, Tokyo, Japan) with 13 MHz linear probe. Resting blood pressure was recorded prior to ultrasound measurements. Blood samples were obtained to quantify triglycerides, HDL cholesterol, LDL cholesterol, total serum cholesterol, high sensitive C-reactive protein (hsCRP), and glucose levels in serum.

Data were analyzed by statistical software package SPSS v.17.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of the values was tested using the Kolmogorov-Smirnov test. Student's t-test and Mann-Whitney U test were applied for the statistical analysis of continuous variables. Multivariate linear regression analysis examined the statistical significance and contribution of the CAL variable in explaining the IMT variance when the following variables were included: age, sex, education, physical activity, smoking, body mass index (BMI), blood pressure, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and hsCRP. The statistical significance was set at  $p < 0,05$ .

## Results

This study examined 57 (44.5%) male and 71 (55.5%) female subjects, divided into four groups.

The largest number of chronic periodontitis patients completed secondary school (64.9%), and were not involved in any kind of physical activity (56.8%), while their controls had an university degree (63.0%), and were engaged in physical activity at least once or twice a week (74.1%). Both groups mainly included nonsmokers, 59.5% and 55.6%, respectively. No significant difference was found in BMI, blood pressure, triglycerides, HDL cholesterol, LDL cholesterol, total serum cholesterol and glucose values. The levels of hsCRP were statistically higher in the test group in relation to the control group (2.2 and 0.7 mg/L, Mann-Whitney test,  $p < 0,005$ ). The comparison of the test and control groups by Mann-Whitney U test revealed a statistically significant difference ( $p < 0,001$ ) for all periodontal indices. The

s kroničnim parodontitisom iznosili su 5,17 i 5,94 milimetra, što je značajno više od 2,79 i 2,9 milimetara u kontrolnoj skupini.

Statistički veće vrijednosti IMT-a pronađene su kod pacijenata s kroničnim parodontitisom (tablica 1.). No, kada smo promatrali pojedinačne vrijednosti, samo je za desnu CCA ustanovljena statistički značajna razlika (tablice 2. i 3.). Kod pacijenata s kroničnim parodontitisom CAL-varijabla nije bila statistički značajan prediktor za ukupne vrijednosti IMT-a u multivarijantnom modelu. Kada se CAL-varijabla promatrala pojedinačno, pokazala se statistički značajnim prediktorom za vrijednosti IMT-a desne CCA (Beta=0,429, t=3,188, p=0,003).

Većina pacijenata s agresivnim parodontitisom i njihove kontrole imali su visoku stručnu spremu (60,0% i 58,8%). Ispitanici u objema skupinama bavili su se fizičkom aktivnošću barem jedanput ili dva puta na tjedan (56,7% i 61,8%) i uglavnom su bili nepušači (53,3% i 67,6%). Značajne razlike nisu nađene za sljedeće vrijednosti: krvni tlak, trigliceride, HDL i LDL kolesterole te ukupni serumski kolesterol. Ispitivana skupina statistički se značajno razlikovala u vrijednostima BMI-a i glukoze (p<0,005). U odnosu na kontrolnu skupinu, ispitivana je imala značajno povišene vrijednosti

median PD and CAL values for chronic periodontitis patients were 5.17 and 5.94 mm, which was significantly higher when compared to 2.79 and 2.9 mm in control group.

Statistically higher values of IMT were found in chronic periodontitis patients (Table 1). However, when values were observed separately, only the right CCA showed statistical significance (Tables 2 and 3). In chronic periodontitis patients group, CAL variable was not a statistically significant predictor for overall IMT values in the multivariate model. When CAL was observed separately, it was found to be a statistically significant predictor only for the right carotid IMT (Beta=0.429, t=3.188, p=0.003).

Most patients with aggressive periodontitis and their controls had a university degree, 60.0% and 58.8%, respectively. Subjects in both groups were mainly engaged in physical activity once or twice a week (56.7% and 61.8%), and did not smoke (53.3% and 67.6%). No significance was found in blood pressure, triglycerides, HDL cholesterol, LDL cholesterol and total serum cholesterol values. The BMI and glucose values were significantly higher (p<0.005) in the test group. The test group had significantly elevated levels of hsCRP compared to the control group (1.4 and 0.6 mg/L, Mann-Whitney test, p<0.005). Among these groups,

**Tablica 1.** Razlike u vrijednostima IMT-a ispitivanih i kontrolnih skupina  
**Table 1** IMT differences between the test and control groups

	Medijan • Median	IQR	p	AUC
Kronični parodontitis • Chronic periodontitis	0.8	0.6-1	0.016	0.37
Kontrola • Control	0.7	0.5-0.8		
Agresivni parodontitis • Aggressive periodontitis	0.5	0.4-0.6	0.453	n/a
Kontrola • Control	0.5	0.4-0.5		

Mann-Whitneyjev U test, IQR=interkvartilni raspon, p=statistička značajnost, AUC= standardna mjera veličine učinka.  
Mann-Whitney U test, IQR=interquartile range, p=statistical significance, AUC= standardized measure of effect size.

**Tablica 2.** Vrijednosti IMT-a lijeve CCA  
**Table 2** IMT of the left CCA

	Medijan • Median	IQR	p	AUC
Kronični parodontitis • Chronic periodontitis	0.8	0.7-1	0.158	n/a
Kontrola • Control	0.8	0.6-0.9		
Agresivni parodontitis • Aggressive periodontitis	0.5	0.4-0.6	0.453	n/a
Kontrola • Control	0.5	0.4-0.5		

Mann-Whitneyjev U test, IQR=interkvartilni raspon, p=statistička značajnost, AUC= standardna mjera veličine učinka.  
Mann-Whitney U test, IQR=interquartile range, p=statistical significance, AUC= standardized measure of effect size.

**Tablica 3.** Vrijednosti IMT-a desne CCA  
**Table 3** IMT of the right CCA

	Medijan • Median	IQR	p	AUC
Kronični parodontitis • Chronic periodontitis	0.8	0.6-1	0.044	0.19
Kontrola • Control	0.7	0.5-0.8		
Agresivni parodontitis • Aggressive periodontitis	0.5	0.4-0.6	0.701	n/a
Kontrola • Control	0.5	0.4-0.5		

Mann-Whitneyjev U test, IQR=interkvartilni raspon, p=statistička značajnost, AUC= standardna mjera veličine učinka.  
Mann-Whitney U test, IQR=interquartile range, p=statistical significance, AUC= standardized measure of effect size.

hsCRP-a (1,4 i 0,6 mg/L, Mann-Whitneyjev test,  $p < 0,005$ ). Skupine su se statistički značajno razlikovale prema svim parodontnim indeksima ( $p < 0,001$ ; Mann-Whitneyjev test). Medijan PD i CAL-vrijednosti kod pacijenata s agresivnim parodontitisom iznosili su 4,73 i 5,17 milimetara, što je mnogo više od 2,60 i 2,62 milimetra u kontrolnoj skupini.

Uspoređujući pacijente s agresivnim parodontitisom i njihove kontrole nisu pronađene statistički značajne razlike u ukupnim vrijednostima IMT-a (tablica 1.), ali ni u pojedinačno promatranim mjerenjima (tablice 2. i 3.). U multivarijantnom modelu jedino je dobivena granična statistička značajnost CAL-varijable za povećane ukupne vrijednosti IMT-a kod pacijenata s agresivnim parodontitisom ( $p = 0,088$ ).

## Rasprava

Parodontitis i ateroskleroza kronične su upalne bolesti. Mnogobrojni dokazi upućuju na povezanost parodontitisa i povećanog rizika za kardiovaskularne bolesti (14). Osim toga, utvrđeni su i zajednički rizični čimbenici tijekom razvoja obje bolesti koji govore u prilog njihovoj neizravnoj povezanosti. Persson i suradnici istaknuli su da je oko 50 posto osoba starijih od 60 godina zahvaćeno parodontitisom i kardiovaskularnom bolešću (15), no utjecaj spola u povezanosti parodontne bolesti i rizika od kardiovaskularnih bolesti i dalje ostaje nejasan (16). Niži stupanj edukacije dokazano je povezan s učestalošću parodontne bolesti te pojavnošću i neželjenim ishodom moždanog udara (17,18). Redovita fizička aktivnost može djelovati protektivno, pa čak i odgoditi povećanje vrijednosti IMT-a karotidnih arterija kod osoba s povišenim krvnim tlakom (19). Za razliku od skupine s agresivnim parodontitisom, većina pacijenata s kroničnim parodontitisom nije se redovito bavila fizičkom aktivnošću i imala je srednju stručnu spremu. Uzevši u obzir njihovu dob, navedeno bi moglo objasniti povećane vrijednosti IMT-a kod starijih ispitanika. Pušenje je jasno prepoznat rizični čimbenik za parodontitis i aterosklerozu. No, ispitivane skupine u našem istraživanju uglavnom su bile sastavljene od nepušača. U ranijim studijama kod nepušača nije bila ustanovljena povezanost parodontitisa i sistemskih bolesti (20).

Mnogobrojni predisponirajući metabolički čimbenici za kardiovaskularne bolesti mogu biti promijenjeni zbog kroničnih infekcija, kao što je parodontitis. Postoje podaci koji govore da uznapredovali parodontitis može utjecati na vrijednosti triglicerida, LDL i HDL kolesterola te glukoze (21). Te se vrijednosti nisu statistički razlikovale između ispitivanih skupina, osim glukoze u krvi (5,14 mmol/L) kod pacijenata s agresivnim parodontitisom. Prema stajalištu Američke udruge dijabetičara (American Diabetes Association), navedene vrijednosti ipak su ispod kritičnih 5,5 mmol/L koje se koriste za dijagnosticiranje predijabetesa.

CRP je utvrđen jetreni marker reakcija akutne faze i može imati uzročnu ulogu u svim fazama ateroskleroze, uključujući disfunkciju epitela, stvaranje i destabilizaciju plaka, te u slučaju aterotrombotičnih komplikacija (22). Prema stajalištu Centra za kontrolu bolesti i prevenciju (Centers for Disease Control and Prevention) te Američke udruge srčanih bolesnika (American Heart Association), vrijednosti hsCRP-a

the Mann-Whitney U test revealed a statistically significant difference ( $p < 0,001$ ) for all periodontal indices. The median PD and CAL values for aggressive periodontitis patients were 4.73 and 5.17 mm, which was significantly higher than 2.60 and 2.62 mm in the control group.

Statistically significant differences between groups with aggressive periodontitis and controls were not found for overall IMT measurements (Table 1), or when values were observed separately (Tables 2 and 3). The multivariate model showed CAL as only a marginally statistically significant predictor for increased overall IMT in aggressive periodontitis patients ( $p = 0,088$ ).

## Discussion

Periodontitis and atherosclerosis are both chronic inflammatory diseases. A growing body of evidence documented an association between periodontitis and elevated risk of cardiovascular disease (14). Furthermore, there are many mutual risk factors in the course of both diseases that support their indirect causal relationship. Persson et al. found that roughly 50% of subjects older than 60 years of age were affected by periodontitis and cardiovascular disease (15), but the role of gender in the relationship between periodontal disease and risk of cardiovascular disease seems to be unclear (16). Lower level of education was shown to be associated with the prevalence of periodontal disease, and the incidence and poor outcomes of stroke (17, 18). Regular physical activity can be protective, or even delay the progression of carotid IMT in hypertensive individuals (19). In contrast to aggressive periodontitis group, the majority of our chronic periodontitis patients was not involved in any kind of regular physical activity, and had a lower level of education. Considering their age, this could contribute to the higher IMT readings among older subjects. Smoking is a well recognized risk factor for both periodontitis and atherosclerosis, but in this research both test groups were mainly comprised of nonsmokers. Similar to the present findings, no periodontitis-systemic disease association has been previously established among nonsmokers (20).

Many metabolic factors that predispose to cardiovascular disease might be altered by chronic infections such as periodontitis. It was suggested that a severe periodontitis can affect serum levels of triglycerides, total cholesterol, LDL, HDL, and blood glucose (21). Those values did not statistically differ between examined groups, except for the fasting glucose levels (5.14 mmol/L) in aggressive periodontitis group. However, according to the American Diabetes Association, those levels were still under the critical value (5.5 mmol/L) accepted for defining pre-diabetes.

CRP is an established hepatocyte-derived marker of acute phase reactions that could be causative in all stages of atherosclerosis, including endothelial dysfunction, plaque formation and destabilization, and atherothrombotic complications (22). According to the Centers for Disease Control and Prevention and the American Heart Association, levels of hsCRP  $< 1$ ,  $1$  to  $3$ , and  $> 3$  mg/L can be used to denote lower,

<1, od 1 do 3, i >3 mg/L mogu poslužiti kako bi se naznačio nizak, umjeren i visok relativni rizik za buduće vaskularne komplikacije. Osim toga, dokazano je da je CRP snažan i neovisan prediktor za infarkt miokarda i moždani udar (23). U našem su istraživanju vrijednosti hsCRP-a bile statistički povećane u objema ispitivanim skupinama, što upućuje na to da je parodontna bolest potaknula sistemske upalne reakcije. Štoviše, zbog vrijednosti od 2,2 i 1,4 mg/L, ispitivani pacijenti s parodontitisom nalaze se u skupini umjerenog rizika za spomenute komplikacije. Inicijalna parodontološka terapija može značajno umanjiti navedeni rizik, što je potvrđeno i u dosadašnjim studijama (24,25).

Istraživanje je pokazalo statistički značajno veće ukupne vrijednosti IMT-a i veće vrijednosti IMT-a desne karotidne arterije samo kod pacijenata s kroničnim parodontitisom. Leivadaros i suradnici istaknuli su da pacijenti s uznapredovalim parodontitisom (u dobi  $\geq 45$  godina) imaju karotidni IMT 0,64 milimetra (0,54 – 0,75 mm), što je u skladu s našim opažanjima. No, nisu našli razlike u vrijednostima IMT-a između kontrolne i ispitivane skupine (26). Naši podaci razlikuju se od rezultata Södera i suradnika koji su dobili vrijednosti IMT-a od 0,66 milimetara i 0,68 milimetara za desnu i lijevu karotidnu arteriju (27). Razlike se mogu tumačiti činjenicom da se ispitanici uključeni u pojedino istraživanje mogu razlikovati prema, primjerice, genetskim čimbenicima, pušenju i prehranbenim navikama. Nakon što su uključeni i ostali rizični čimbenici u multivarijantni model, CAL-varijabla se pokazala statistički značajnim prediktorom samo za IMT desne karotidne arterije. Treba uzeti u obzir da ostali kardiovaskularni rizični čimbenici, poput dobi i hsCRP-a, mogu znatno utjecati na aterosklerotične promjene u starijoj populaciji.

U proteklom razdoblju istraživanja vrijednosti IMT-a karotidnih arterija uglavnom su se obavljala ako je pacijent s parodontitisom bio srednje ili starije dobi, pa stoga nedostaju podaci o povezanosti parodontne bolesti i ateroskleroze kod mlađih pacijenata s agresivnim parodontitisom. Agresivni parodontitis rjeđi je oblik parodontne bolesti i najčešće zahvaća mlađu populaciju s rapidnom destrukcijom potpornih tkiva zuba. Premda se taj oblik može pojaviti u bilo kojoj dobi, obično je ograničen na osobe mlađe od 35 godina (28). U istraživanju Cairoa i suradnika ustanovljeno je da je uznapredovali parodontitis povezan sa supkliničkom aterosklerozom kod mlađih ( $\leq 40$  godina) sistemski zdravih pacijenata (29). Ukupne vrijednosti IMT-a iznosile su 0,82 milimetra i 0,72 milimetra za ispitivanu i kontrolnu skupinu, što je u suprotnosti s našim vrijednostima od 0,5 milimetara za obje skupine. Postoji i mogućnost da su stvarne vrijednosti IMT-a bile podcijenjene jer je dokazano da IMT karotidnih arterija može varirati ovisno o različitom stupnju istezanja arterijske stijenke (30). U multivarijantnom modelu nije ustanovljena statistička značajnost CAL-varijable za povećane ukupne vrijednosti IMT-a kod pacijenata s agresivnim parodontitisom. S obzirom na to da su i ostali zajednički kardiovaskularni rizični čimbenici manje izraženi u mlađoj populaciji, možda bi osjetljivija mjerenja, poput određivanja arterijske elastičnosti (31), mogla pokazati potencijalnu ulogu parodontitisa u najranijim fazama ateroskleroze.

average and higher relative risk for future vascular events. It is proven to be a strong, independent predictor of myocardial infarction and stroke (23). In the present study, the levels of hsCRP were statistically higher in both test groups, which may indicate that periodontal disease contributed to the systemic inflammatory responses in our subjects. Moreover, due to the values of 2.2 and 1.4 mg/L, our periodontitis patients run an average relative risk for the abovementioned events. That risk can be significantly reduced by means of non-surgical periodontal treatment, as reported in previous studies (24, 25).

This research revealed significantly higher values of overall and right carotid IMT only in chronic periodontitis patients, compared with periodontally healthy subjects. Leivadaros et al. found that severe periodontitis patients ( $\geq 45$  years) had a carotid IMT of 0.64 mm (0.54-0.75 mm) which is in agreement with our observations. However, they reported no difference in carotid IMT between the control and periodontitis group (26). Our data do not concur with the results published by Söder et al., who found 0.66 mm and 0.68 mm for the right and left IMT, respectively (27). This may be explained by the fact that different genetic factors, smoking and dietary habits can vary among subjects included in the particular study. CAL variable was a statistically significant predictor only for the right carotid IMT in the multivariate model, when potentially confounding factors were included. It should be noted that other cardiovascular risk factors, such as age and CRP, strongly correlate with atherosclerotic changes in the older population.

In the recent period, papers have usually evaluated carotid IMT in middle-aged to elderly periodontitis patients, so that there are limited data concerning the association between periodontal disease and atherosclerosis in young population with aggressive periodontitis. Aggressive periodontitis is a less common type of periodontitis that generally affects young patients with rapid destruction of tooth supporting tissues. Although this form can occur at any age, it was usually confined to individuals under the arbitrarily chosen age of 35 years (28). In a study from 2008, Cairo et al. showed that severe periodontitis is associated with sub-clinical atherosclerosis in young ( $\leq 40$  years) systemically healthy patients (29). The overall mean carotid IMT was 0.82 mm in the test group and 0.72 mm in the control group, which was not in accordance with our results of 0.5 mm found in both groups. There is always a possibility that true IMTs were underestimated, as it was proven that the carotid IMT may vary depending on varying degree of stretching of the arterial wall (30). The multivariate model showed that CAL variable was not a statistically significant predictor for IMT values in aggressive periodontitis patients. Since common cardiovascular risk factors are less manifested in the younger population, a more sensitive measurement such as arterial stiffness assessment (31) might therefore reveal a potential role of periodontitis in the earliest stages of atherosclerosis.

Aminbakhsh et al. reviewed available information on IMT measurements in healthy subjects and created a framework that can be used to define an abnormal result. They concluded that the risk of first myocardial infarction increas-

Aminbakhsh i suradnici pregledali su dostupne podatke o mjerenjima IMT-a kod zdrave populacije i izradili okvir prema kojem se definiraju patološke vrijednosti. Zaključili su da se rizik od prvog infarkta miokarda povećava nakon vrijednosti IMT-a od  $\geq 0,822$  milimetra, a opasnost od moždanog udara nakon vrijednosti IMT-a od  $\geq 0,75$  milimetara (32). Ako uzmemo u obzir interkvartilne raspone IMT-a kod ispitivanih pacijenata s kroničnim parodontitisom, očito je da oni značajno prelaze navedene kritične vrijednosti IMT-a povezane s rizikom od koronarnih i cerebrovaskularnih komplikacija. Za razliku od dosadašnjih istraživanja (29), pacijenti s agresivnim parodontitisom ne pripadaju rizičnoj skupini za spomenute komplikacije.

### Zaključak

Dobiveni rezultati jasno su pokazali da aterosklerotične promjene karotidnih arterija mogu biti povezane s kroničnim parodontitisom. Potrebna su daljnja mikrobiološka, imunološka, biokemijska i genetička istraživanja na većem uzorku pacijenata kako bi se utvrdio utjecaj agresivnog parodontitisa na vrijednosti IMT-a karotidnih arterija. Također se preporučuje da se pacijenti s uznapredovalim oblicima parodontitisa upute na ultrazvučni pregled karotidnih arterija.

### Zahvala

Istraživanje je rađeno u sklopu projekata 065-0650444-0415 (*Sistemni aspekti u etiologiji parodontnih bolesti*, voditelj Darije Plančak) i 134-1340036-0034 (*Funkcionalna dijagnostika moždane cirkulacije*, voditelj Arijana Lovrenčić-Huzjan) Ministarstva znanosti, obrazovanja i sporta Republike Hrvatske.

### Izjava

Autori opovrgavaju bilo kakav sukob interesa.

es with an IMT of  $\geq 0.822$  mm, and the risk of stroke with an IMT of  $\geq 0.75$  mm (32). If we consider the IMT interquartile ranges in chronic periodontitis patients, it is clear that they considerably overcame those critical IMT thresholds related to the risk of coronary and cerebrovascular events. According to this study, and opposed to the previous findings (29), our aggressive periodontitis patients were not in the risk group for such events.

### Conclusion

The present results clearly demonstrated that carotid atherosclerotic changes can be linked with chronic periodontitis. Further and larger-scale studies on microbiological, immunological, biochemical and genetic levels are required to more precisely assess the influence of aggressive periodontitis on carotid IMT. It is also advisable that the patients with severe form of periodontitis are referred to the carotid artery ultrasound.

### Acknowledgements

This paper was supported by the Ministry of Science, Education and Sport of the Republic of Croatia Grant No. 065-0650444-0415 (project leader Darije Plančak, project "Systemic aspects in the etiology of periodontal diseases"), and Grant No. 134-1340036-0034 (project leader Arijana Lovrenčić-Huzjan, project "Functional diagnostics of cerebral circulation").

### Transparency declaration

The authors deny any conflicts of interest.

### Abstract

**Objective:** The objective of this study was to investigate the relationship between carotid intima-media thickness (IMT) and periodontal status in periodontitis patients. **Materials and methods:** The study was conducted on 128 subjects divided into four groups. The test groups consisted of 37 patients with chronic periodontitis (18 men and 19 women, mean age  $57.2 \pm 8.0$  years) and 30 patients with aggressive periodontitis (13 men and 17 women, mean age  $34.7 \pm 6.3$  years). There were 27 subjects in the control group for chronic periodontitis (11 men and 16 women, mean age  $53.0 \pm 10.1$  years), and 34 subjects in the control group for aggressive periodontitis (15 men and 19 women, mean age  $27.7 \pm 5.7$  years). Both control groups consisted of periodontally healthy volunteers. Periodontal condition was assessed using periodontal indices. Triglycerides, HDL cholesterol, LDL cholesterol, total serum cholesterol, high sensitive C-reactive protein (hsCRP), and glucose levels were quantified in serum. IMT measurements on common carotid artery were performed using Aloka ProSound ALPHA 10 with 13 MHz linear probe. **Results:** This research revealed significantly higher values of overall (0.8 mm) and right (0.8 mm) carotid IMT in chronic periodontitis patients only. Chronic periodontitis was a statistically significant predictor ( $p=0,003$ ) for the right carotid IMT in the multivariate model, when potentially confounding factors were included. Both periodontitis groups mainly included nonsmokers, and had statistically higher levels of hsCRP (2.2 and 1.4 mg/L). **Conclusions:** The study demonstrated that chronic periodontitis can be linked with carotid atherosclerosis, but further research is required for a more precise assessment of the influence of aggressive periodontitis on carotid IMT.

**Received:** August 19, 2012

**Accepted:** October 10, 2012

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### Key words

Periodontitis; Periodontal Index; Carotid Stenosis; Tunica Intima; C-Reactive Protein

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