

INFLAMMATORY BIOMARKERS AS POTENTIAL MEDIATORS FOR THE ASSOCIATION BETWEEN PERIODONTAL AND SYSTEMIC DISEASE IN KOSOVO

Zana Sllamniku-Dalipi^{1*}, Fatmir Dragidella¹, Metush Disha¹, Kastriot Meqa¹,
Luljeta Begolli², Gramos Begolli²

1. Department of Periodontology and Oral Medicine, School of Dentistry, Medical Faculty, University of Prishtina, Prishtina, Republic of Kosovo

2. Department of Biochemistry, Medical Faculty, University of Prishtina, Prishtina, Republic of Kosovo

Abstract

The possibility of a connection between oral cavity and systemic diseases has been intensively researched in recent years. The aim of this study was to compare the serum levels of high-sensitivity C reactive protein (hs-CRP), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α) between patients with and without periodontitis.

Clinical periodontal parameters: gingival index, the dental plaque index, the probing depth, the clinical attachment level, the bleeding index and the tooth mobility index were measured at six sites per tooth in seventy-five subjects with periodontitis and in thirty-five periodontally healthy subjects. Serum levels of hs-CRP and inflammatory cytokines were assessed via Enzyme-Linked ImmunoSorbent Assay. Inter-group significance was determined by the Student's t-test, x²-test and Mann-Whitney test.

The concentrations of each biomarker (control group vs. periodontal disease group) were as follows: hs-CRP (0.5 \pm 0.6 vs. 2.5 \pm 2.6, respectively), IL-1 β (2.1 \pm 2.2 vs. 7.0 \pm 11.6, respectively), IL-6 (1.9 \pm 1.6 vs. 3.7 \pm 4.4, respectively) and TNF- α (64.6 \pm 72.3 vs. 80.0 \pm 73.1, respectively). The differences between the groups showed statistical significance at p < 0.05.

Periodontal disease was associated with increased circulating concentrations of hs-CRP and inflammatory cytokines.

Clinical article (J Int Dent Med Res 2013; 6: (1), pp. 1-5)

Keywords: Periodontal disease, interleukin-1 beta, interleukin-6, tumour necrosis factor alpha, high sensitivity- C reactive protein.

Received date: 27 January 2013

Accept date: 12 February 2013

Introduction

Periodontitis is an infectious disease resulting in the inflammation of gingival and periodontal tissues and the progressive loss of alveolar bone. It has also been considered a risk for a variety of systemic conditions, including cardiovascular disease, diabetes mellitus, rheumatoid arthritis, and respiratory disorders.¹ The link between periodontal inflammation and

systemic health has yet to be fully elucidated; although, a low-grade inflammatory burden has been proposed as a possible biologic mechanism linking periodontitis and the abovementioned conditions.² During periodontitis, bacteria and their metabolic by-products stimulate a local cellular immune response represented by a dense infiltration of neutrophils, macrophages and other lymphoid cells. These cells, and the connective tissue cells of the host associated with the inflammatory lesion, are thereby stimulated, resulting in the synthesis and release of the following proinflammatory cytokines and prostanoids: interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin 8, tumour necrosis factor-alpha (TNF- α), prostaglandin E₂ and various matrix metalloproteinase's. Their vascular distribution via the vascular circulatory system induces the

*Corresponding author:

Dr. Zana Sllamniku- Dalipi
Lagja e Spitalit street, NN, 10000 Prishtina, Republic of Kosovo
Tel: +377 44 222 403
Fax: +381 38 512 474
e-mail: zanasllamniku@hotmail.com

prediction of liver-derived markers of a systemic inflammatory reaction, such as C-reactive protein (CRP).³

The excessive release of inflammatory cytokines in diseased periodontal tissues contributes to the overall pool of serum cytokines, which promotes systemic diseases.

Many studies have compared circulating IL-6 and CRP concentrations between periodontal patients and controls, with several studies showing higher circulating serum levels of CRP, IL-1, and IL-6 in patients with severe periodontitis than in unaffected control populations.^{4,5}

The effects of destructive periodontal disease status, severity and progression on the components of the acute phase response have also been determined. The results of these studies suggest that destructive periodontal disease and disease progression are associated with changes in various serum components consistent with an acute phase response. CRP levels were observed to increase with disease progression.⁶

Elevated circulating levels of TNF- α , IL-6 and CRP have been correlated with an increased risk of the development of cardiovascular diseases and other systemic disorders.^{7, 8} Because severe periodontitis has been associated with increased serum levels of TNF- α , IL-1 β , IL-6 and CRP, a role for periodontal diseases as potential contributors of general inflammation and development of systemic disease has been suggested.^{9,10}

The aim of this study was to compare the serum levels of high-sensitivity C – reactive protein (hs-CRP), IL-1 β , IL-6 and TNF- α between periodontitis patients and periodontally healthy subjects and to assess the degree to which periodontal injury relates to inflammatory mediator levels.

Methods and Materials

This pilot study recruited 110 subjects referred to the Department of Periodontology and Oral Medicine in University Dentistry Clinical Center of Kosovo.

The following patients were excluded from this study: those with known systemic disease, those with history of infective disease, those on systemic antibiotic treatment in the preceding 3 months, those receiving treatment with any

medications known to affect the serum levels of inflammatory markers, pregnant or lactating females, those receiving periodontal treatment within the preceding 6 months and those with fewer than 10 teeth in the mouth.

All patients gave written informed consent. The study had been reviewed and approved by the University Dentistry Clinical Center of Kosovo Joint Ethics Committee (no 1551).

The periodontal assessments were conducted by two calibrated periodontists (ZSD and KM) with an inter-examiner reliability of kappa = 0.88. The two periodontists also collected complete medical histories of all patients.

A full-mouth periodontal examination was conducted and the following variables were determined: gingival index (Löe-Sillness) [11], plaque index (Sillness-Löe) [12], probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP).

The gingival index (Löe-Silness) was recorded for all present teeth as follows: 0 - normal gingival; 1 - mild inflammation, slight change in colour, slight oedema and no bleeding on palpation; 2 - moderate inflammation, moderate redness, moderate oedema, and moderate ulceration and the tendency for spontaneous bleeding; and 3 - severe inflammation, marked redness, marked oedema, and marked ulceration and the tendency for spontaneous bleeding.

The plaque index (Silness and Löe) was recorded for all present teeth as follows: 0 - no plaque in the gingival area; 1 - a film of plaque adhering to the free gingival margin and adjacent areas of the tooth, with plaque recognised only by running a probe across the tooth surface; 2 - a moderate accumulation of soft deposits within the gingival pocket, on the gingival margin, and on the adjacent tooth surface that was observable by the naked eye; and 3 – an abundance of soft deposits within the gingival pocket and/or in the gingival margin as well as on the adjacent tooth surface.

The clinical attachment level (CAL) was measured using the cemento-enamel junction as a reference point, specifically from the cemento-enamel junction to a probable pocket depth.

The probing pocket depth was measured from the crest of the gingival margin to a probable pocket depth.

The probing depth and attachment level

measurements were made at the mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual positions of every tooth, with the exception of third molars.

Bleeding on probing was assessed on the 6 sites at which PD was determined and deemed positive if it occurred within 15 seconds after probing. BOP was expressed as the percentage of sites that showed bleeding.

The presence of 4 or more teeth with 1 or more sites with PD \geq 4 mm and with CAL \geq 3 mm at the same site was diagnosed as periodontal disease [13]. Patients were assigned to 2 categories: those with periodontal disease (n = 75) and those without periodontal disease (n = 35).

Analyses of serum CRP, IL-1 β , IL-6 and TNF- α

A total of 10 ml blood was obtained from each participating subjects by venipuncture using tubes without anticoagulant and immediately transferred for centrifugation. All samples were centrifuged for 10 minutes at 2,000 rpm, and transferred to 1.7-ml Eppendorf tubes and stored at -20C. Serum samples were assessed using a kit with a specific high sensitivity methodology-ELISA test, according to the manufacturer's instructions (IBL International GmbH, Hamburg, Germany).

Statistical analysis

Statistical analysis was performed using the statistical package R. Differences by age group were tested using the Student's t-test of mean values. The other demographic parameters were assessed with the χ^2 test. The differences in clinical periodontal parameters, TNF- α , IL-1 β , IL-6 and hs-CRP levels between periodontitis patients and periodontally healthy subjects were compared using the Mann-Whitney test. Differences were considered significant when $p < 0.05$.

RESULTS

Study population characteristics

One hundred-fifteen patients aged from 20 to 71 were enrolled in this study. They were divided into two groups: a first group of thirty-five

periodontally healthy subjects and a second group of seventy-five patients with periodontitis.

The demographic characteristics of the control and study subjects are shown in Table 1. The mean age of the healthy subjects was 30.4 ± 5.7 years, while subjects with periodontitis had a mean age 45.1 ± 10.7 years. This difference was statistically significant ($P < 0.0001$). Other demographic parameters showed no significant differences ($P > 0.05$).

	Healthy n=35	Periodontitis n=75	P-value
Age (years, mean \pm SD)	30.4 \pm 5.7	45.1 \pm 10.7	$P < 0.0001^*$
Gender, N (%)			
F	18 (51.4)	45 (60.0)	$P = 0.522^{**}$
M	17 (48.6)	30 (40.0)	
Residence, N (%)			
Urban	26 (74.3)	50 (66.7)	$P = 0.559^{**}$
Rural	9 (25.7)	25 (33.3)	
Education, N (%)			
Non	1 (2.9)	2 (2.7)	$P = 0.07^{**}$
Primary	6 (17.1)	8 (10.7)	
Secondary	20 (57.1)	58 (77.3)	
High	8 (22.9)	7 (9.3)	
Smoking, N (%)			
No	20 (57.1)	31 (41.3)	$P = 0.179^{**}$
Yes	15 (42.9)	44 (58.7)	

*significant difference (student T-test), ** no significant difference (χ^2 -test)

Table 1. Study population demographics

Clinical periodontal parameters

The mean values of the periodontal parameters are shown in Table 2. There were significant differences between healthy and periodontitis patients with regard to the plaque index (1.6 ± 0.7 vs. 2.0 ± 0.7 , respectively), calculus index (1.4 ± 0.6 vs. 1.8 ± 0.4 , respectively), bleeding index (24.3 ± 33.0 vs. 76.9 ± 31.0 , respectively), gingival index (0.9 ± 0.6 vs. 1.8 ± 0.5 , respectively), periodontal pocket depth (1.0 ± 0.4 vs. 3.1 ± 0.7 , respectively), gingival recession (0.3 ± 0.5 vs. 1.5 ± 1.0 , respectively), clinical attachment level (1.0 ± 0.7 vs. 4.5 ± 5.9 , respectively), tooth mobility index (0.2 ± 0.5 vs. 1.1 ± 0.9 , respectively), and missing teeth (5.1 ± 5.1 vs. 7.3 ± 5.2 , respectively).

Comparisons between healthy and periodontitis patients with regard to inflammatory markers

Statistical analyses of the protein levels in

patients with and without periodontitis are shown in Table 3. There was a high level of statistically significant differences ($P < 0.0001$) between healthy subjects and those with periodontitis with regard to the levels of hs-CRP (0.5 ± 0.6 vs. 2.5 ± 2.6 , respectively) and IL-1 β (2.1 ± 2.1 vs. 7.0 ± 11.6 , respectively). The mean serum levels of TNF- α were significantly higher in the periodontitis group than in healthy subjects (80.0 ± 73.1 vs. 64.6 ± 72.3 , respectively; $P=0.0128$). Additionally, the mean serum levels of IL-6 showed a statistically significant difference between healthy subjects and periodontitis patients (1.9 ± 1.6 vs. 3.7 ± 4.4 respectively; $P = 0.0318$).

	Healthy n=35	Periodontitis n=75	P-value
Plaque index			
Mean \pm SD	1.6 \pm 0.7	2.0 \pm 0.7	$P=0.0173^*$
Range	0.5 - 3	0.5 - 3	
Calculus index			
Mean \pm SD	1.4 \pm 0.6	1.8 \pm 0.4	$P=0.0014^*$
Range	0.5 - 2.7	0.8 - 2.9	
Bleeding index			
Mean \pm SD	24.3 \pm 33.0	76.9 \pm 31.0	$P<0.0001^*$
Range	5 - 110	10 - 100	
Gingival index			
Mean \pm SD	0.9 \pm 0.6	1.8 \pm 0.5	$P<0.0001^*$
Range	0.1 - 2	0.2 - 3	
Periodontal pocket depth			
Mean \pm SD	1.0 \pm 0.4	3.1 \pm 0.7	$P<0.0001^*$
Range	0.2 - 2.3	1.2 - 4.8	
Gingival recession (mm)			
Mean \pm SD	0.3 \pm 0.5	1.5 \pm 1.0	$P<0.0001^*$
Range	0 - 2	0 - 6.1	
Clinical attachment level (mm)			
Mean \pm SD	1.0 - 0.7	4.5 - 5.9	$P<0.0001^*$
Range	0.1 - 4.3	1.5 - 5.3	
Tooth mobility index (0-3)			
Mean \pm SD	0.2 \pm 0.5	1.1 \pm 0.9	$P<0.0001^*$
Range	0 - 1.5	0 - 3	
Missing teeth			
Mean \pm SD	5.1 \pm 5.1	7.3 \pm 5.2	$P=0.0123^*$
Range	0 - 18	0 - 18	

*significant difference (Mann-Whitney test)

Table 2. Periodontal parameters

	Healthy n=35	Periodontitis n=75	P-value
CRP (μg/ml)			
Mean \pm SD	0.5 \pm 0.6	2.5 \pm 2.6	$P<0.0001^*$
Range	0 - 3.1	0 - 14.1	
Interleukine 1 (μg/ml)			
Mean \pm SD	2.1 \pm 2.2	7.0 \pm 11.6	$P<0.0001^*$
Range	0.2 - 7.4	1 - 56.2	
Interleukine 6 (μg/ml)			
Mean \pm SD	1.9 \pm 1.6	3.7 \pm 4.4	$P=0.0318^*$
Range	0.4 - 6	0.2 - 22.2	
TNF - alfa (μg/ml)			
Mean \pm SD	64.6 \pm 72.3	80.0 \pm 73.1	$P=0.0128^*$
Range	14 - 319	12.8 - 321	

*significant difference (Mann-Whitney test)

Table 3. Systemic levels of inflammatory markers

DISCUSSION

The goals of the present study were to compare the mean levels of TNF- α , IL-1 β , IL-6 and hs-CRP in serum samples from periodontally healthy subjects and subjects with periodontitis to evaluate possible correlations among these biomarkers and clinical parameters of periodontal disease. The results of this study demonstrated that individuals with no history of systemic disease had increased circulating levels of proinflammatory cytokines and hs-CRP. These proinflammatory mediators have been implicated in an increased risk of systemic diseases.^{14,15} The relationship between periodontal disease and CRP has been shown in previous cross-sectional studies.^{16,17} Some of these studies utilised the latex agglutination assay (the best assay available at the time) to report on the presence or absence of CRP¹⁸ at a minimum requirement >10 mg/L for the detection of serum CRP. More recent studies have used more sensitive assays with detection limits as low as 0.21 mg/L. Loss et al.¹⁹ investigated systemic levels of inflammatory markers of cardiovascular disease (e.g., hs-CRP, IL-6, total leukocyte count and differential leukocyte count) in an unselected population both with and without periodontitis, where the disease was diagnosed according to the radiographic evidence of bone loss. Detectable hs-CRP was observed in 90% of all patients. A consistent association between the occurrence of periodontitis and elevated serum levels of CRP in cross-sectional studies was reported by Paraskevas et al.,²⁰ which is consistent with the results of our study. Subjects with periodontitis had significantly higher levels of IL-1 β than did periodontally healthy subjects. These findings reinforce previous reports^{21, 22} that observed associations between the levels of IL-1 β and clinical signs of periodontitis, although different methodologies were used to measure GCG levels. Marcaccini et al.²³ reported a statistically significant difference between control and periodontal disease groups only for plasma IL-6 concentrations ($p = 0.006$); these authors did not observe differences in hs-CRP, and in their hands the hs-CRP levels decreased 3 months after non-surgical periodontal therapy in patients with periodontal disease. Some recent studies have shown that IL-6 and CRP levels are elevated with periodontal disease alone or with other systemic diseases²⁴ in comparison to

healthy groups.

It is important to note that this was the first study conducted in Kosovo estimating serum levels of hs-CRP, IL-1 β , IL-6 and TNF- α between periodontitis patients and healthy patients. Our study provides general information regarding the periodontal status of the population in Kosovo and explored the connection between periodontitis and inflammatory markers. However, we were unable to explore variations between different racial and/or ethnic groups, as has been done in other studies²⁵, because our population is racially and ethnically homogenous.

In this study using a Kosovo population, we show that periodontal disease is associated with increased circulating concentrations of TNF- α , IL-1 β , IL-6 and hs-CRP. Long-term studies assessing the effect of periodontal therapy on levels of TNF- α , IL-1 β , IL-6 and hs-CRP are warranted to further explore the impact of periodontal therapy and to enable the practical management of at-risk patients.

References

1. Williams RC, Offenbacher S: Periodontal medicine: The emergency of a new branch of periodontology. *Periodontol* 2000;23:9-12.
2. Ebersole JL, Machen RL, Steffen MJ, Willmann DE: Systemic acute-phase reactants, C-reactive protein and haptoglobin in adult periodontitis. *Clin Exp Immunol* 1997; 107(2):347-352.
3. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E: Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol* 2001; 72(9):1221-1227.
4. Kinnane DF, Lappin DF: Immune processes in periodontal disease : A review. *Ann Periodontol* 2001;7:62-71.
5. Buhlin K, Gustafsson A, Pockley AG, Frostegard J, Klinge B: Risk factors for cardiovascular disease in patients with periodontitis. *Eur Heart J* 2003; 24:2099-2107.
6. Craig RG, Yip JK, So MK, Boylan RJ, Socransky SS, Haffajee AD. Relationship of destructive periodontal disease to the acute- phase response. *J Periodontol* 2003;74:1007-1016.
7. Borden EC, Chin P: Interleukin 6: a cytokine with potential diagnostic and therapeutic roles. *Journal of Laboratory and Clinical Medicine* 1994;123:824-829.
8. Bradley JR: TNF- mediated inflammatory disease. *J Pathol* 2008;214:149-160.
9. Ebersole JL, Machen RL, Steffen MJ, Willmann DE: Systemic acute-phase reactants, C-reactive protein and haptoglobin in adult periodontitis. *Clin Exp Immunol* 1997; 107:347-352.
10. Loos BG: Systemic markers of inflammation in periodontitis. *Journal of Periodontology* 2005;76:2106-2115.
11. Loe H: The gingival index, the plaque index and the retention index systems. *J Periodontol* 1967;38:610.
12. Silness P, Loe H: Periodontal disease in pregnancy. *Acta Odontol Scand* 1964;22:121.
13. Lopez NJ, Smith PC, Gutierrez S: Periodontal therapy may reduce risk of preterm low birth weight in women with periodontal disease: a randomized controlled trial. *J Periodontology* 2001; 73:911-924.
14. Ridker PM, Morrow DA: C-reactive protein, inflammation, and coronary risk. *Cardiol Clin* 2003;21:315-325.
15. Beckman JA, Preis O, Ridker PM, Gerhard Herman M: Comparison of usefulness of inflammatory markers in patients with versus without peripheral arterial disease in predicting adverse cardiovascular outcomes (myocardial infarction, stroke and death). *Am J Cardiol* 2005;96:1374-1378.
16. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E: Periodontal infection contribute to elevated systemic C-reactive protein level. *J Periodontol* 2001;72:1221-7.
17. Boucher NE Jr, Hanrahan JJ, Kihara FY: Occurrence of C-reactive protein in oral disease. *J Dent Res* 1967;46:624.
18. Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S: Relationship between periodontal disease and C reactive protein among adults in the Atherosclerosis Risk in Communities study. *Arch Intern Med* 2003;163:1172-9.
19. Loos BG, Craandijk J, Hoek FJ, Wertheim –van Dillen PM, van der Velden U: Evaluation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000; 71:1528-34.
20. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008;35:277-290.
21. Engebreston SP, Lamster IB, Herrera- Abreu M, Celenti RS, Timms JM, Chaudhar AGA, Giovine F, Kornman KS: The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *J Periodontol* 1999;70:567-573.
22. Hou LT, Liu CM, Rossomando EF: Cervicular interleukin 1 beta in moderate and severe periodontitis patients and the effect of phase I periodontal treatment. *J Clin Periodontol* 1995;22:162-167.
23. Marcaccini AM, Meschiari CA, Sorgi, Saraiva MCP, De Souza AM, Faccioli LH, Tanus-Santos J, Novaes AB, Gerlach RF: Circulating interleukin-6 and high-sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. *J Periodontol* 2009;80:594-602.
24. Persson GR, Pettersson T, Ohlsson O, Renvert S: High-sensitivity serum C-reactive protein levels in subjects with or without myocardial infarction or periodontitis. *J Clin Periodontol* 2005;32:219-224.
25. Albert MA: Race/ethnicity and inflammation- is there a link with periodontal disease? *J Periodontol* 2008;79:1121-3.